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SIR WILLIAM OSLER AND PARASITOLOGY*

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Thirty years ago today, William Osler died in Oxford, England. During his long and active life he had played the major part in initiating the greatest revolution in the teaching and practice of medicine—both human and veterinary; he had joined the laboratory to the clinic to help end empiricism and he, probably more than anyone else, had appreciated the full significance of the Theory of Evolution as it affected man himself.

William Osler was born in 1849 in a country parsonage at the edge of the great Canadian forest which in those days extended to within a few miles of Toronto.

When he was ten years old, Darwin's THE ORIGIN OF SPECIES was published but it was six years later before he began to come under its influence. It was then he went to Weston School, near Toronto, and became a pupil of the Rev. William Arthur Johnson. Johnson was born in India but in his early days lived at Down House in England (where Darwin later lived and died). He came to Canada in 1831 and founded the school at Weston which young Osler attended. Johnson was not only an artist; he was an enthusiastic microscopist, with an intense love for nature, and his enthusiasm soon infected Osler. Within a year the lad had become an ardent collector and an excellent technician. Johnson's love of natural history soon brought him into contact with Dr. James Bovell of Trinity College, Toronto, an equally enthusiastic naturalist.

James Bovell was a West Indian who had studied medicine in London, Edinburgh, and Glasgow, subsequently returned to the West Indies and later migrated to Canada. In Toronto in 1850, he helped organize the Trinity College Medical School, becoming its first Dean and Professor of the Institutes of Medicine. However, the school was short-lived although Bovell remained as Professor of Natural Theology in the College, and became Professor of Medicine at the Toronto Medical School.

Dr. Bovell had very close contacts with Father Johnson and it was natural that young Osler, when he went to college in 1867, should come under Bovell's influence. Osler had originally planned to enter the church but he changed his mind and commenced to read for a medical degree at the the Toronto Medical School in 1868. The microscope was still very prominent in the young man's mind and it was used with increasing frequency for subjects akin to medicine, especially the Entozoa. On the 1st January, 1870, Osler began to keep a note-book of the helminths he had

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collected from various sources and this note-book is still extant. At this time, it is obvious he was well on the way to becoming a parasitologist and, as Harvey Cushing comments in his biography, "there can be little doubt that had William Osler at this time come under the influence of Leidy or Agassiz or, possibly, of Huxley, he would have gone on with his biological studies and abandoned medicine."

However, such influence was absent while that of James Bovell prevailed. Perhaps some significance should also be attached to the proximity of Griffith Evans—a medical graduate of McGill—who was the veterinary officer attached to the Artillery Regiment stationed at Toronto. Evans, like his friend Bovell, was an enthusiastic microscopist and later, of course, was to become famous as the discoverer of the trypanosome parasite which causes surra in India.

In 1870, James Bovell returned to the West Indies and Osler left Toronto for McGill University, Montreal. He never escaped from Bovell's naturalist viewpoint but it was henceforth to be modified by that of Dr. Palmer Howard, Professor of Medicine in McGill.

McGill in the seventies was undoubtedly the best medical school in Canada—possibly in North America; its only serious rival being Philadelphia (where Osler went in 1884). Howard was a hard master and young Osler had to spend much time on clinical work but still he found time to collect parasites—not only from rats he picked up in the General Hospital, but from fish from the market and dead animals from the local Natural History Society, as the entries in his note-book show.

The Toronto-McGill periods are really one insofar as Entozoa are concerned and we may conveniently diverge here to examine his note-book and papers. Many of the specimens mentioned in his note-book were sent to the Rev. W. A. Johnson and cross-reference between Osler's and Johnson's records are of considerable interest. Thus on the 21st April the note-book reads:

> "21/IV/70 On the fins of chub in the Rev. W. A. Johnson's aquarium were noticed several round white spots. These on examination proved to be some sort of Entozoa. In addition to these, some yellow spots were seen which seem to be a more advanced condition of the parasite."

While in Johnson's note-book under the following day's date, appears the entry:

> "Parasites on fins, body, etc. of little fish in my aquarium. They seem to have a chitinous horse-shoe shaped piece inside and are large brown-looking things with powers of locomotion and short cells all around the edges. Gelatinous mass destroyed by boiling."

There are many other references to fish parasites, thus:

> "23/IV/70 In a pike 2 ft. 7 in. long caught at the Island, I obtained 68 specimens of Taenia and two or three small Ascaridae.
>
> "This tape-worm is about a foot long, and exhibited curious undulatory movements which continued for more than twenty-four hours after removal from the intestines. It is very extensible, and may be stretched to almost double its ordinary length. The head is flattened, club-shaped when the worm is dead, but during life is generally extended, giving to it the shape of a flint arrow-head. Five suctorial disks are plainly seen but no hooklets. The segments taper very gradually, being exceedingly small at the neck, larger towards the end of the body, they are about twice as broad as they are long. The water vascular system is most distinctly seen in this worm, consisting of four channels, two on each side. At the head and for a considerable distance down the neck, these tubes connect by means of inosculating branches, those about the head form a dense net-work."
>
> "13/V/70 From four Perch caught in the canal I obtained the following:
> "A Cysticercus from the liver, the head of which bears a remarkable resemblance to the head of the Taenia of the pike and most probably is its scolex. Encysted nematodes from the liver.

"Four species of Echinorhynchus from the rectum.
"In the intestine there was a curious entozoon with a bell-shaped retractile head furnished with small finger-like processes.
"Coiled around the base of the aorta was a large distoma. The upper third is outlined in this sketch."

"3/VI/70 From the intestine of a Pickerel in the fishmarket I obtained 8 Taenia. This is a comparatively stout Cestode, length from 10–12 inches, breadth of posterior segments about ¼ of an inch. Head square, no neck, but a gradual tapering to the head. disks four, large, no hooklets observed."

"22/X/70 Examined a dorey in Montreal fish market. One small tape-worm, one Bothriocephalus and about two dozen small Echinorhynchidae were found. The Bothriocephalus occupied the entire cavity of one of the numerous caeca given off at the pylorus. The Taenia was in the duodenum and the Echinorhynchidae move towards the rectum. This fish I find is the Pickerel."

"4/VII/70 Examined ten Sun-fish caught in the Canal; in all numerous Distomes were found. The fluke is probably an immature condition, being encysted and not having its internal organs completely developed. The heart, liver and kidneys presented a swollen appearance from the numbers in them. They seemed only to be attached to the heart, while in the liver and kidneys they occupied the substance of those organs.
"Encysted Nematodes were found in one liver and what appeared to me the scolex of one of the Echinorhynchidae, in another.
"In the rectum of two, a few Echinorhynchidae were found."

However, he was interested in more than fish and his best known fresh-water parasite came from the water-puppy. Here is his own description from his notebook:

"6/VI/70 From a specimen of the above, caught at the Island I obtained numerous Polystomes, some of which were attached to the branchiae, others to the upper surface of the mouth. This Trematode presents four disks, two at each extremity. Of these the smaller one situated about the middle of the upper fourth of the body, is the mouth, which leads directly into the intestines. These consist of two simple tubes, which unite about the middle of the lower fourth of the body. The water vascular system is well developed. It appears to commence in a ramification of vessels about the anterior disk, these unite to form two vessels, which run the whole length of the body, join below and open somewhere between the posterior disks. Cilia are to be seen distinctly in the water vascular system, especially at the junction of the tubes below. At the upper third of the body on a level with the generative opening, on each side is seen a curious pulsating organ which is undoubtedly connected with the water vascular system. The pulsations occur about every minute and a half. The external generative orifice is seen a little below the oval disk, this leads into a narrow, slightly curved vagina. The ova occupy the general cavity of the body between the digestive tubes. A bundle of spicules (Penis?) are to be seen close to the vulva. Close to the caudal disks two large hooks are placed. These hooks are bifurcate and to one extremity a band of fibres is attached. Besides these numerous small hooklets are to be seen scattered about the posterior disks. The attached extremity of these is trifurcate."

However, nothing was done about this until some years later when, after graduation, he was home for Christmas in 1874 and met Ramsey Wright, newly arrived Professor of Biology at Toronto. He presented him with these polystomes but Ramsey Wright did not describe them until 1879, when he wrote:

"I have lately received from my friend Professor Osler of Montreal, several specimens of a worm taken from the gills and cavity of the mouth of our common lake lizard. . . . From some specimens, in a good state of preservation, mounted by Dr. Osler for microscopic examination, and also from his notes and sketches made on observation of the fresh specimens, I am able to communicate the following . . ."

These specimens were named *Sphyranura osleri* nov. gen. et spec.

Osler never altogether lost his interest in this group of peculiar worms, as the following post-card of his to Dr. Horace W. Stunkard shows:

"3-X-18
13, Norham Gardens,
Oxford

"Many thanks for your polystome monograph received today. I have left enough knowledge of the subject to appreciate your good work. R. R. Wright is now living in Oxford and will be interested in it.
"With greetings and regards, Wm. Osler."

He was equally interested in mammals and there are several references to human parasites in the pages of the note-book. In the winter of 1870 he pointed out the presence of trichina cysts in one of the bodies under dissection in the anatomy laboratory—a fact none of his fellow students had observed but which was curiously reminiscent of Paget's similar observations in 1835 in a London dissecting room. Osler referred to his own discovery six years later in a paper written from Montreal:

". . . we discovered numerous trichinae throughout the whole muscular system, all of which were densely encysted, many having become calcified. From a single drachm of one of the muscles of the arm I obtained 159 cysts, the greater number of which enclosed healthy-looking worms. This man was a German and had been janitor at the hospital, where I had known him for over two years."

This case is referred to in his note-book as Case IV, and he apparently tried feeding experiments with it as the following entry shows:

"From a dog about a year old which had on a former occasion (1/3/70) been fed with flesh from Case IV, 4 Ascaris and five Taenia elliptica, all with heads, were obtained. No Trichinae were found either in the intestines or in the muscles, but on examining the kidneys, six or seven small white spots were seen about 0, that size; in each of these a small nematode worm was observed, not coiled up, and looking exceedingly like a Trichina spiralis."

He was more successful with another experiment a month or so later. "The parasites in this case were not so old, none of them beginning to undergo degeneration." These were fed to a pup and a rabbit: the pup was negative when examined a fortnight later but in the rabbit (killed three weeks after infection):

"numerous trichinae in a young immature condition were observed in the muscles. Many of them exhibited sluggish movements. They were more numerous in the abdominal and thigh muscles than in any other."

However, Osler's first two records of trichina were earlier:

"7/II/68 Trichina spiralis. Obtained a piece of muscle from a man who died of the disease in Illinois State, very numerous, not encysted. Two families living on different flats of the same house bought a barrel of pork between them. One flat cooked the pork and escaped the disease, the other ate theirs raw and nine were sickened; of these nine, four died."
"27/II/69 The family of a Mr. Getz in Hamilton consisting of himself, wife and daughter partook of an uncooked ham. All three were laid up with the disease. Miss Getz died first; in her the parasites were numerous and unencysted. Mrs. Getz died some two weeks after her daughter; in her they were just beginning to be encysted. The husband was attacked but not so severely and escaped, most probably from being drunk for some days at the commencement of the attack."

He was equally interested in tapeworms, thus:

"23/II/71 Taenia mediocanellata. Obtained three specimens of this cestode from a man (Earle) who died of heart disease in Montreal General Hospital. He stated during life that he had been suffering from tapeworm for 14 years, got at Malta while stationed there with his regiment. He stated that he had been under treatment for it innumerable times and had passed many yards of the worm. While in Hospital during the end of last year and beginning of this, he was treated with the male shield fern, which brought away several long portions. On opening the small intestine the worms were found extending from the lower part of the duodenum through the jejunum and seemed com-

pletely to fill the intestine in the empty condition. The heads were all within 1½ inches of each other and deeply imbedded in the mucous membrane, between the valvulae conniventes. The bodies were convoluted and twisted, extending down the intestine for about 3½ feet, and lower down in the bowel several detached portions were found, consisting of from six to eighteen proglottides. The worms still retained some little vitality, but the movements were very feeble. While in the intestine, the water-vascular system in one of them was beautifully seen, extending up each side of the worm. The length was of each respectively 76, 50 and 65 inches, and the number of segments amounted in each to between 275 and 350, falling at least 100 short in the longest one of the fully mature sexual segments. This however is not to be wondered at as it is not more than six weeks ago since he passed several yards. The chief differences between this form and T. solium appears to be as follows: The head is larger, abruptly terminated, and lacks the rostellum and consequently the hooklets. It is surrounded by a dark zone of calcareus corpuscles which form a striking contrast to the white segments of the neck. The segments are broader, thicker and not as long. The generative orifice is a little below the centre, and the lateral branches of the uterus appear more numerous and closely packed together. There appears also to be a difference in the shape of the ova in the two species, that in T. solium being round, while in T. marginata (marginata is certainly a lapsus for mediocanellata) they are rather oval and somewhat larger."

He examined all kinds of mammals—squirrels, rats, bats, pigs, a lynx and even a skunk.

"15/VI/70 From a large male skunk, about 30 Taenias and 14 Ascaridae. Numerous small cysts were observed in the liver and spleen but nothing found in them. The tapeworms are small, from ½ an inch to 2 inches in length, broad in proportion, exhibiting very slight movements. A slight enlargement seems to exist about the neck, which disappears when the worm is much elongated. The segments seem but loosely joined together, breaking very easily. Four larger sucking disks exist at the head, no hooklets seen. The calcareous corpuscles are more numerous in this cestode than in any I have yet examined. The water vascular system is not easily seen on account of the dense layer of calc. corpuscles.

"The Ascaridae are from ¾ of an inch to one inch in length. They move freely. Most of them were in the stomach, not in the intestines."

The ascarids are most probably *Ascaris columnaris* originally described by Leidy. The entry is of considerable personal interest because the first paper on helminths which I wrote (in collaboration with Dr. T. Goodey) was on the morphology and life history of this same parasite.

Meanwhile other interests were clamouring for his attention. He graduated from McGill in 1872 and immediately proceeded to Britain and the Continent. This was a time of searching of soul for the young man—he thought of the Indian Medical Service, he considered becoming an oculist, he worked under Leuckart and other celebrated German and Austrian medical scientists, but finally settled down in London to the study of physiology and experimental pathology. (During his time in London, he lived in Gower Street, that long unlovely street, as Tennyson called it, which now houses, *inter alia,* the London School of Hygiene and Tropical Medicine, one of the magnificent Rockefeller gifts to Preventive Medicine. It was at this period too that his interest in the colourless blood cells began and he made his celebrated discovery of the blood platelets; this interest was the preface to his later work on Malaria.)

In 1874, Osler returned to Canada and was appointed lecturer on the Institutes of Medicine—an old Edinburgh term for physiology, histology and pathology—but physiology in its older inclusive meaning. The following year he became Professor, taking over also the pathological laboratory at the Montreal General Hospital, and introducing the microscope and the laboratory into the course on the

"Institutes of Medicine." Incidentally, he paid for the first dozen microscopes with a $600 fee received for undertaking work in the smallpox wards.

However, he was by no means ready to abandon helminthology although it was becoming more and more of a medical nature.

Early in 1876 he addressed the Montreal Natural History Society on "Animal Parasites and their Relation to Public Health" and the following year commenced his lectures on parasites at McEachran's Montreal Veterinary School. He was Professor of Physiology there as well as a Vice-President of the local Veterinary Medical Association. (Later this school was absorbed into McGill as the Faculty of Comparative Medicine—Osler not only suggested the name, but continued as lecturer on the "Institutes of Medicine.")

In this same year he described canine broncho-pneumonia caused by a new nematode which he called *Strongylus canis-bronchialis,* and which was renamed two years later *Filaria osleri* by Cobbold and, in 1921, *Oslerus osleri* by Maurice Hall. He also wrote papers on hydatid disease and on parasites of the Montreal pork supply, while a few years later, he demonstrated amphistomes and verminous aneurysms before the local Medical Society and with the help of one of his students (A. E. Clement), made an exhaustive study of trichina, measles and hydatid in local pigs. In 1882 he presented a paper on hydatid disease in America to the Canadian Medical Association at Toronto. This paper was a statistical account of 61 cases drawn from various sources and Osler signed himself "Lecturer on Helminthology, Montreal Veterinary College."

In the summer of 1884, on a visit to Berlin, he records having

> "spent two afternoons a week at the abattoir, which owing to the elaborate system of inspection, both ante- and post-mortem, offers one of the best fields in Europe for the study of comparative pathology and helminthology."

However, his active helminthological period was really over and in the fall, aged 35, he left Canada for Philadelphia. As Councilman ten years later said:

> "He could easily have become a great scientist but he chose the path which led to the formation of the great clinician which he became."

His parasitological interests were gradually shifting towards the more minute forms and while these studies reached their maximum during his American period, they had begun in Canada. In 1881 he had written a paper describing trypanosomes in the blood of frogs and in 1882, at the A.A.A.S. meeting in Montreal (under the Presidency of the Principal of McGill University and before the Histology Section), he read three papers on blood—including demonstrations of phagocytosis of red cells by leucocytes, although he did not use the term which was invented by Metchnikoff four years later.

In 1884 Osler went to the University of Pennsylvania, where for five years, he occupied the chair of Clinical Medicine. During this period he made many enduring friendships—including one with the aging Leidy who (then 61 years of age) was the leading spirit of the Biological Club—a dining club of extremely distinguished men, which met twice monthly to talk. It was also during his Pennsylvania period that Laveran's parasite became a subject of controversy. Councilman (from Welch's laboratory in Baltimore) introduced the subject at a meeting in Washington in June of 1886 and Osler put on record the fact that he also had

observed amoeboid bodies—and drawn them too—in malaria but was not yet inclined to regard them as parasites. However, he returned immediately to Philadephia and took up the search anew. In July he was well on the way to conviction and, by October, he was converted to Laveran's belief. His drawings show he had seen both crescents and flagellation. Osler regarded the flagellate forms as the "adult" parasites, a misconception which was later cleared up by McCallum at Johns Hopkins when working with avian malaria. Osler was an immediate convert to the parasite theory of malaria—at a time when few believed and believers, such as Manson, were regarded as unsound. So convinced, however, was Osler, that very shortly, a blood smear became mandatory in his clinics for all cases suspected of being malaria.

In 1889 he became the first Physician-in-Chief at the Johns Hopkins Hospital with William Welch, first Professor of Pathology, as one of his colleagues.

To his Johns Hopkins' period belong his studies on *Entamoeba histolytica*. These organisms, first described by Loesch in 1875 in St. Petersburg in Russia, were not regarded seriously until about 1886, when Kartulis published his observations on dysentery in Egypt. Even then, however, little attention was at first paid to the importance of the parasite in Europe or America, until Osler reported them in a patient from Panama. On March 22nd, 1890, he spent many hours watching an amoeba under the microscope and making drawings of the changes it underwent; these drawings are still in his note-book in the Osler library at McGill. The amoebae came from a liver abscess—the first Anglo-Saxon confirmation of the disputed records of Kartulis. These observations quickly led to local search and the parasite was found to be common in Baltimore. It has since been declared to be the commonest human infection in North America.

However, even the excitement of malaria and amoebae did not completely extinguish his interest in Metazoa. Shortly after his arrival at Baltimore, we find him studying *Filaria sanguineus-hominis* and in 1896 one of his students (Dr. T. R. Brown) reported the discovery of eosinophilia in trichinosis—a result of Osler's insistence on his senior undergraduates making close microscopical examination of the blood of their patients.

In 1905 Osler went to Oxford as Professor of Medicine in England's oldest university. He had previously in 1900 rejected an appeal to apply for the Chair of Medicine at Edinburgh—then the leading British medical school—an application which would inevitably have meant appointment: in the despondency following a bout of 'flu he had a dread of the cold gloominess of the northern city. (Osler made some amends for this by standing for the Rectorship of Edinburgh in 1908. He was one of three candidates, the other two being Winston Churchill and George Wyndham; Wyndham was elected by the students by a narrow margin, a sorry commentary on the ability of university undergradutes to form enduring judgments.) At Oxford, he was now medical consultant, the organizer, the teacher, the administrator—a tremendous influence in all things medical both in Britain and North America—he spent almost every summer there and during his life must have crossed the Atlantic at least two dozen times.

In 1907 he wrote from Oxford to Professor Robertson in Montreal about the establishment of a Department of Medical Zoology at McGill, suggesting that Stiles might be called upon to direct it: His letter reads:

"13 Norham Gardens, Oxford,
February 3rd, 1907.

"Dear Professor Robertson,

"When in Montreal a few weeks ago I had a chat with Sir William Macdonald and Mr. Peterson on the possibility of organizing, in connection with the Agricultural College, an extensive department of medical zoology in which the whole subject of Parasitism should be considered. Sir William was anxious that I should see you, but I had only part of two days in Montreal. I promised him to get a scheme from Stiles of Washington, who is certainly the leading expert on parasites in the English-speaking world. The Department could be made a most important one and it has such close affiliations with disease that the same man could very well lecture on parasites in the medical school. There would be no lack of candidates for such a place, and there are one or two very good men available, particularly Todd who had done so much good work on the Ticks. I should not be surprised, however, if such a position were thrown open, that Stiles himself might be a candidate. I have asked Stiles to prepare a memorandum which I will forward to you.

Sincerely yours,
Wm. Osler."

Stiles prepared this memorandum and I have examined it in detail elsewhere. The department was ultimately formed as the Institute of Parasitology but not until 25 years later.

Nevertheless, Osler's greatest contribution to parasitology was indirect and unwitting. It arose from the effects of the second edition of his "Principles and Practice of Medicine" on F. T. Gates who read it during his summer vacation in the Catskills in 1897. It made a profound impression on him, and he persuaded Mr. Rockefeller and his son that Osler's frank disclosure of medicine's limitations in 1897 was a challenge to research. As a consequence the Rockefeller Institute of Medical Research came into being on the fourteenth of June, 1901. The following year, a million dollars was given to Harvard and, after a series of comparatively minor donations, on the 3rd March, 1919, John D. Rockefeller, Sr., announced his stupendous gift "for the promotion and dissemination of knowledge; the prevention and relief of suffering."

Of a more personal nature was his influence on the magnificent Nuffield gifts to Oxford to further medical science. Osler was one of the earliest car owners in that city and often met young Morris in his bicycle shop, where matters relating to medicine and medical research were freely discussed. The Professor's free and unpretentious manner and his obvious conviction as to what could be done, so impressed Morris that he never forgot; later, when he became the wealthy Lord Nuffield, he still remembered. The medical headquarters of the Nuffield Foundation at Oxford is named Osler House.

Osler's Canadian period was one of devotion to research and to the laying, on natural history, of the firm foundation which stood him in such good stead in later years. His Philadelphia-Baltimore period was basically one of clinical development and of clinical teaching. His Oxford period was one rather of social medicine and preparing the way for the future.

All who have worked in the field of Parasitology have felt his influence and this is equally true for those who have tried to apply the findings in the laboratory to practice in the field. There is no better example of this than the case of William Crawford Gorgas, the greatest of tropical sanitarians. In Osler's own words "there is nothing to match the work of Gorgas in the history of human achievement." His greatest achievement was the successful building of the Panama Canal in the face

of malaria and yellow fever. Osler had a considerable share of responsibility in this: "I know that to a man," he wrote, "the profession in the United States felt that could Dr. Gorgas be given full control of the sanitary affairs of the Panama zone, the health problem, which meant the Canal problem, would be solved. There was at first a serious difficulty relating to the necessary administrative control by a sanitary officer. In an interview which Dr. Welch and I had with President Roosevelt, he keenly felt this difficulty and promised to do his best to have it rectified." It was rectified and Gorgas was placed in charge. As Dr. Paul Russell pointed out a few years ago:

> "It is easy to forget and time rapidly dims even the brightest records. Hence it does not seem amiss to recall once more the tremendous worldwide impression made by the sanitary victories in Havana and Panama. For instance, when Gorgas visited London in 1914, he received, according to Osler, the greatest ovation ever given a medical man in England." (Osler was, as a matter of fact, largely responsible for this also.) "At home, the President made him Surgeon General of the Army, and the Congress about a year later made him a Major General, at that time an almost unprecedented rank for a medical officer. He served his country with distinction in this high office during the World War . . .
> "In 1920, in London, Gorgas became ill and was taken to the Queen Alexandra Military Hospital. There he was knighted by George V, receiving from the King's hand the insignia of Knight Commander of the Most Distinguished Order of St. Michael and St. George. A few days after receiving this knighthood, Gorgas died and was given the funeral of a British Major General in St. Paul's Cathedral, the highest honor that Britain could bestow."

On General Gorgas' casket in London, lay a solitary wreath—that sent by Sir Patrick Manson. The two men had never met, yet General Gorgas' widow wrote to Lady Manson:

> "The work my husband accomplished in yellow fever and malaria was founded on the discoveries of your husband, Sir Patrick Manson. The world will not forget him and the benefits of his work every generation will know and appreciate. Dr. Gorgas yielded to no man in his love and admiration for Sir Patrick."

Osler was the foremost representative of Medicine in the Biological Renaissance which occurred early last century and he was one of the few who really understood the significance of Darwin's theory of evolution. His great part in the evolution of modern medicine was firmly to anchor the art of practice to the science of the laboratory—and this must have been due to his early work on Entozoa while still a student. He preached the end of empiricism; he was, as he expressed it himself, a therapeutic nihilist. He preached also prevention rather than cure and advocated persistently the widest reading and searching of other men's minds for knowledge. His whole approach was that of the evolutionist. He played the part in medicine in temperate climates that Manson played in tropical medicine. The two men were about the same age (Osler was in fact five years younger) and both had very similar philosophies. Both were great clinicians as well as diagnosticians of the highest order, but both had an abiding interest in parasitology—it was an accident which took one to the tropics and kept one in north temperate climates. Both had a wide interest in disease and both were firm believers in the great importance of comparative medicine and the study of disease processes in animals. Both could think ahead and both had the gift of attracting young men who would continue their work into the future. Both were great teachers who left their ideas firmly impressed on modern medical education, and it is no coincidence that the two men

most favored for the Oxford Chair of Medicine in 1905 were Osler and Manson. It is perhaps idle to speculate as to what would have happened had Manson rather than Osler been appointed—probably very little would have changed, because both had the same strong missionary spirit which made their service to the world almost identical—they were the catalysers who revivified medical practice in temperate climates and in warm lands. Both were great naturalists who, under the influence of the basic fundamental truth of Darwinism that all animals are related and that man is an animal, were able to change the blind anthropological gropings of neo-medieval scholasticism into the scientific approach of modern medicine.

In the first edition of his "Tropical Diseases" Manson wrote:

> "It is evident from what has been advanced that the student of medicine must be a naturalist before he can hope to become a scientific epidemiologist or pathologist or a capable practitioner. The necessity for this in all departments of medicine is yearly becoming more apparent, but especially so in that section of medicine which relates to tropical disease."

There is no preface in the first edition of Osler's "Principles and Practice of Medicine" but he expressed exactly the same sentiment when he dedicated it to William Arthur Johnson, James Bovell, and Robert Palmer Howard.

PARASITIC INFECTIONS AMONG NATIVES OF THE NORTH MARKHAM AREA, NEW GUINEA[1]

HOWARD A. BERN[2] AND MERLE F. HANSEN[3]

This paper is the result of a series of surveys conducted in the North Markham area, New Guinea, from May to July 1944, in an attempt to determine the incidence of parasitic infections in natives of this area. In view of recent consideration of the possibility of settling the northeastern New Guinea region (Bowman, 1948), it is felt that these data may be of some current interest. The villages surveyed are located in the flat valley north of the Markham River and along the Erap River, a tributary of the Markham, 30 to 40 miles inland from Lae. The accompanying map (fig. 1) shows the approximate relative locations of these villages, which are

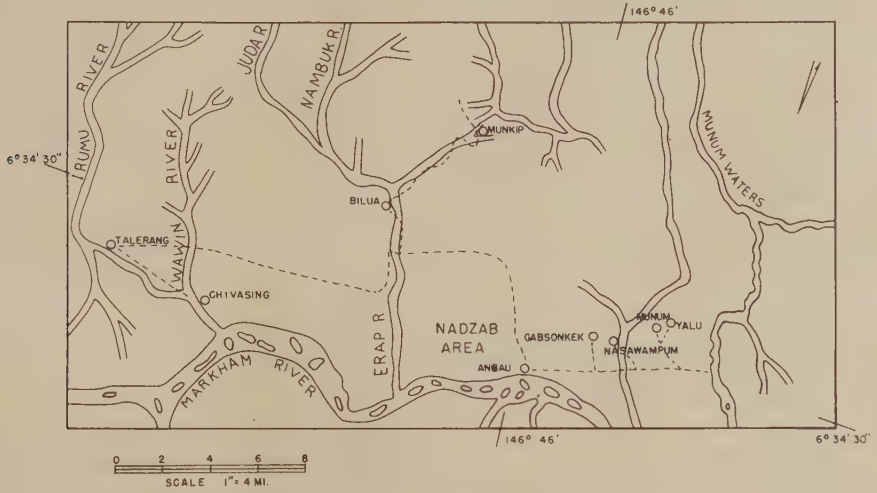

FIG. 1. Map showing approximate relative location of native villages surveyed in the North Markham area, New Guinea. Detachment was based in Nadzab.

all situated in the area suggested by Bowman (1948) as a possible site for agricultural settlement.

OBSERVATIONS

Malaria. The Markham Valley was highly malarious, all three species of *Plasmodium* being well represented. Table 1 shows the incidence of malaria in the vari-

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[1] The data reported herein were collected during the authors' tours of duty as parasitologists with the 32nd Malaria Survey Detachment, Fifth Air Force, A.U.S., under the command of Karl V. Krombein. The able technical assistance of Millard Ross, Alfred Salerno and Franklin Honicker, former Medical Department laboratory technicians, and the valuable cooperation of Mr. Nolan of ANGAU, Nadzab, New Guinea, are gratefully acknowledged. The authors are also indebted to Professor Harold Kirby, Department of Zoology, University of California, for his advice on publication of this material.

The Addendum, written by Karl V. Krombein, Division of Insects, U. S. National Museum, Washington, D. C., lists the mosquito vectors of malaria and filariasis in the Nadzab area, New Guinea.

[2] University of California.

[3] University of Kentucky.

ous villages. Subtotals are given to indicate the incidence for adults and children, as well as for the population as a whole. Results of a survey conducted on ANGAU native laborers (all male) at Nadzab are also included. Although these natives came from all over the Markham region, they had been in the Nadzab area for approximately one year.

One thick and one thin blood smear were taken during the day (1200–1500) from each individual and stained with dilute Giemsa's stain. Whenever possible, the species was determined on the thin smear. Adrenalin was administered to all adults except the ANGAU laborers: 0.5 ml. of 0.1 per cent adrenalin salt solution subcutaneously one hour before smears were taken. Comparison of results on a group of laborers with and without adrenalin injections indicated that the incidence would have been 3–4 per cent higher than that reported for the ANGAU natives in table I, if adrenalin had been administered prior to taking the smear.

TABLE 1.—*Incidence of malaria in native villages*

Village	Number examined	Parasite index (%)	Gametocyte index (%)	*P. vivax* (%)	*P. malariae* (%)	*P. falciparum* (%)	*P. sp.* (%)	Double infection
CHIVASING								
Total Pop.	111	42	8	7	11	21	4	One: *P.v.* & *P.f.*
Children	57	61	14	12	14	31	5	
Adults	54	20	2	2	7	9	2	
MUNUM								
Total Pop.	85	38	5	4	11	20	5	One: *P.v.* & *P.f.*
Children	52	48	4	6	10	31	4	
Adults	33	21	6	0	12	3	6	
TALERANG								
Total Pop.	47	43	4	6	34	2	2	One: *P.v.* & *P.f.*
Children	18	72	11	11	56	6	6	
Adults	29	24	0	4	21	0	0	
NASAWAMPUM								
Total Pop.	62	52	8	15	19	8	10	
Children	23	74	17	30	35	4	4	
Adults	39	39	3	5	10	10	13	
GABSONKEK								
Total Pop.	57	39	2	12	9	7	11	
Children	11	64	9	27	9	18	9	
Adults	46	33	0	9	9	4	11	
MUNKIP								
Total Pop.	39	49	14	31	8	5	8	One: *P.v.* & *P.m.*
Children	15	73	33	67	13	0	0	
Adults	24	33	0	8	4	4	17	
BILUA								
Total Pop.	99	32	4	18	7	5	4	Two: *P.v.* & *P.f.*
Children	41	56	10	37	15	7	2	
Adults	58	16	0	5	2	3	5	
AVERAGE 7 VILLAGES								
Total Pop.	500	40.6	6.0	12.0	12.8	11.2	5.8	1.2%
Children	217	60.4	11.9	21.6	18.5	18.9	4.1	2.8%
Adults	283	25.4	1.4	4.6	8.5	5.2	7.1	0.0%
Men	83	25.4	2.4	1.2	8.5	7.2	8.5	0.0%
Women	200	25.5	1.0	6.0	8.5	4.5	6.5	0.0%
ANGAU LABOR CAMP, NADZAB								
Adult males	526	10.8	1.7	4.0	1.6	2.3	2.9	0.0%

The three major species of *Plasmodium* were equally prevalent in the area as a whole, although in different villages different species predominated. Children, on an average 60.4 per cent positive on the basis of a single smear, can be expected to

have been almost all malarious, as was indicated by splenic indices. In addition, children were the primary gametocyte carriers. The parasitic index for the male laborers was considerably lower than that for village males; this was probably due to fairly effective mosquito control in the labor camp area.

Filariasis. These data are based primarily on the examination of thick smears taken from 184 native laborers at 2300 during the month of June. The nocturnal incidence of microfilariae in this group was 30.0 per cent. The species is *Wuchereria bancrofti.* Although this species in New Guinea has a nocturnal microfilarial periodicity, a low diurnal incidence was found in adults (table 2). Several

TABLE 2.—*Daytime incidence of microfilariae in natives*

Group	Number of adults examined	Number positive	Daytime incidence
			%
Native laborers	491	11	2.2
Chivasing adults	54	2	3.7
Munum adults	33	0	0.0
Talerang adults	29	0	0.0
Nasawampum adults	39	1	2.6
Gabsonkek adults	46	0	0.0
Munkip adults	24	0	0.0
Bilua adults	58	3*	5.2
TOTALS	774	17	2.2

* These three were women; all other positives were men.

cases of elephantiasis were seen in the native villages (including one of the scrotum), but, on the whole, Mr. Nolan of ANGAU Native Hospital at Nadzab had found elephantiasis to be quite rare.

Intestinal Helminthiasis. Fecal specimens from ANGAU laborers and village natives were examined for helminth ova and larvae using the zinc sulfate flotation technic. The native populations examined represent a mixture of adults and children.

TABLE 3.—*Incidence of intestinal helminths in natives*

Group	Number examined	Hookworm	*T. trichiura*	*A. lumbricoides*	*S. stercoralis*	Multiple infection
		%	%	%	%	%
Native laborers	167	91	18	5	1	17
Chivasing	101	77	3	4	5	8
Munum-Yalu	35	90	20	6	6	20
Talerang	50	68	2	2	2	8
Nasawampum	28	82	7	4	4	14
Gabsonkek	33	76	0	0	6	3
Bilua	49	55	10	6	0	8
TOTALS	463	80	10	4	3	12

Hookworm is a serious problem in this area, as can be seen from table 3. The low ascarid incidence is somewhat surprising. Although a few specimens were positive for *Enterobius vermicularis* ova, the flotation technic employed does not give an accurate picture of this infection. Among individuals with multiple infections, several showed all four species of helminths listed in table 3.

DISCUSSION

The malaria parasitic index of 50.3 per cent reported by Avery (1946) for the Samarai natives of Papua is higher than the Markham overall average of 40.6 per cent and twice as high as the adult native index of 25.4 per cent. However,

the seriousness of the malaria problem, indicated by Bowman (1948), is self-evident. The Markham natives showed considerably higher *P. falciparum* and lower *P. vivax* indices than the Samarai natives. The Markham nocturnal microfilarial incidence of 30.0 per cent reported herein parallels Avery's finding of a 30.5 per cent incidence in the Samarai district. The severity of the filariasis problem should not be underestimated in any proposals for settlement of the Markham valley.

The hookworm incidence of 80 per cent is about the same as that reported by Burrows (1945) for a mixed Papuan-Indonesian-Chinese population from the Vogelkop Peninsula of Dutch New Guinea and by Avery (1946) for the Samarai natives. Both Burrows and Avery found a considerably higher frequency of whipworm and Burrows of roundworm than are reported herein.

SUMMARY

1. Parasitism among natives of the North Markham area, New Guinea, was surveyed in mid-1944.

2. The malaria parasite index was 40.6 per cent with a gametocyte index of 6.0 per cent. The three major species of *Plasmodium* were represented about equally in the total village population.

3. *Wuchereria bancrofti* was indicated by a nocturnal incidence of 30.0 per cent in a group of 184 native laborers and by a diurnal incidence of 2.2 per cent.

4. Hookworm was found in 80 per cent of the total population examined; whereas whipworm, roundworm and strongyloid infections were encountered in 10 per cent, 4 per cent and 3 per cent of the natives, respectively.

ADDENDUM

Several members of the *Anopheles punctulatus* group, which are considered to be the important vectors of malaria in New Guinea, were collected commonly in the Nadzab area. The larvae of these species breed in shallow side pools of streams and shallow depressions containing standing water such as wheel ruts. Since the species prefer a sunny habitat, the clearing operations incidental to agricultural activities would undoubtedly create additional areas favorable for breeding.

Of the large list of mosquitoes known to transmit filariasis in the East Indies, three were collected commonly in the Nadzab area—*Aedes* (*Stegomyia*) *scutellaris* (Walker), *Culex* (*Culex*) *annulirostris* Skuse and *Mansonia* (*Mansonioides*) *uniformis* (Theobald). The first two species are widely distributed over the entire Nadzab area; the larvae breed in a variety of situations including artificial containers, wheel ruts, coconut shells, and *A. scutellaris* also in banana leaf axils and even large fallen leaves on the ground. *Mansonia* was found only in swampy areas along streams draining into the Markham River. Its larvae remain submerged below the surface and obtain air by inserting the air tube into the hollow stems of aquatic plants, obviously posing a very difficult control problem.

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THE PROTOZOAN FAUNA OF SOME SPECIES OF INTERTIDAL INVERTEBRATES IN SOUTHERN CALIFORNIA

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This paper is chiefly a report of a survey conducted of the protozoan fauna of ten marine species of gastropods collected in the vicinity of the University of California at Los Angeles. The ten gastropod species belong in the groups commonly termed limpets and turbans, of the order PROSOBRANCHIATA, suborder SCUTIBRANCHIATA. The ten host species surveyed fall into two divisions: (1) Of the division DOCOGLOSSA, the species are *Acmaea pelta, A. digitalis, A. limatula, A. scabra, A. insessa, A. fenestrata fenestrata,* and *Lottia gigantea,* all in the family ACMAEIDAE (the limpets), (Grant, 1938; Oldroyd, 1927). (2) Division RHIPIDOGLOSSA, subdivision ZYGOBRANCHIATA, *Fissurella volcano* (the volcano limpet), and subdivision AZYGOBRANCHIATA, *Tegula funebralis,* and *T. ligulata,* the black and speckled turbans, respectively, of the family TROCHIDAE (Oldroyd, 1927; Pratt, 1935). Eight hundred and fifty-nine animals in all were examined and their contents noted.

Interest was primarily focused upon the ciliate population when it was found that it comprised the consistent bulk of the parasite faunules present within the host species. The ciliates were found to be of six different species. Four were species of the order PERITRICHA, *Urceolaria karyolobia* Hirshfield (1949), *Urceolaria korschelti* Zick (1928), *Trichodina tegula* Hirshfield (1949), of the family URCEOLARIIDAE, and *Scyphidia ubiquita* Hirshfield (1949), of the family SCYPHIDIIDAE. The morphology of *Urceolaria karyolobia, Trichodina tegula,* and *Scyphidia ubiquita* is described in another paper (Hirshfield, 1949). The fifth was a heterotrich of the family LICNOPHORIIDAE, *Licnophora conklini* Stevens (1904), and the sixth a thigmotrich of the family ANCISTRUMIDAE, *Eupoterion pernix* MacLennan and Connell (1931). This study is the report of the incidence of the above six ciliates within the ten host species and cross infection experiments conducted between the host species.

COLLECTION SITES AND METHODS OF COLLECTION

The host organisms were collected either in buckets or isolated at the collection sites in stoppered jars or vials. They were collected principally from three sites. From north to south these areas are: (1) Big Rock Beach near Topanga Canyon, approximately six miles north of Santa Monica. This is a rocky area with one large prominent boulder, from which the name is derived. The second site (2) was the wooden pilings of the abandoned Crystal Pier, about one-half mile south of the Santa Monica Pier, and approximately six and one-half miles south of Big Rock Beach. (3) The third area, Flat Rock Beach, is located on the Palos Verdes Estates about sixteen miles south of Santa Monica. This again is a large rocky beach, much greater in extent than Big Rock Beach. (4) One collection was made from the concrete bridge and wooden pilings at Playa del Rey, which is about five

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[1] This paper is from a thesis presented to the Graduate School of the University of California at Los Angeles in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The thesis, containing the complete tables of all original data, is in the Zoology Library, U.C.L.A., Los Angeles, California.

miles south of Santa Monica. The host organisms were maintained without difficulty in aquaria aerated, at 18° C.

HOST SYSTEMATICS

The species of the genus *Acmaea* were determined as closely as possible by the use of shell and radular characteristics. Close correlation was kept between the inquiline population of an individual host and the host. In the case of the few hybrids found, the inquiline data were at first separated; however, when it was later found that the differences between the various host species of *Acmaea,* as regards the parasite populations, were quantitative rather than qualitative, the data from the hybrids were placed into the more obvious parent species.

In all instances the host population was sampled as randomly as possible as to size, sex, or location. Due to the large number of species examined, the numbers of individuals of each species were limited.

INCIDENCE STUDIES

A. Location of the parasites within the host.

Eupoterion pernix was found within the gut of the infected hosts.

All of the other five protozoa were found within the mantle and nuchal cavities of the host organisms. The *Scyphidia* were found attached to the edge of the mantle, or ctenidium (pallial or mantle gill) in large numbers. The *Urceolaria, Licnophora* and *Trichodina* were present in highest numbers in the nuchal cavity, a space above the "head" within which lies a small ctenidium. The urceolarids occupied niches in the ctenidium itself, pushing themselves into spaces between gill filaments, and seemed firmly imbedded.

B. Effect of the parasites upon the host.

The parasites seem to have little or no effect upon the hosts. The primary food of the protozoa are bacteria which swarm in the mantle cavity and upon which the protozoa feed heavily. The possibility of decreasing respiratory surfaces in very heavy infections seems possible, but opposed to this is the increase in incidence of parasites in the more vigorous hosts.

There is a direct relationship between the health of the host and the incidence of the parasites, as has been pointed out previously. Thus in diseased or failing hosts the incidence of the parasites dwindles to the vanishing point to be replaced by other ciliates, e.g., *Colpoda, Euplotes,* etc., which swarm in tremendous numbers once the host dies. The six forms studied disappear almost completely from the moribund host.

C. Incidence in other hosts.

A cursory survey was made of other intertidal gastropods for the six protozoa studied. A few specimens of *Megathura crenulata,* the giant key-hole limpet, and *Haliotis cracherodii,* the black abalone, both related to the limpets, were examined and found to be completely negative for all six protozoans. A few *Crepidula onyx,* the black slipper shell, were examined and likewise found negative.

Acanthina spirata and *Norrissia norrissii* were successfully infected with *Scyphidia* and *Urceolaria karyolobia* in the laboratory. Neither, however, were ever

found infected with these forms in nature, but only a very few specimens of each species were examined. One *Tegula aureotincta,* the golden turban, was found infected in nature with *Scyphidia.*

Examination of invertebrates of other phyla, such as sea anemones, shore crabs, barnacles, annelids, failed to yield any of the six forms described in this study.

D. Discussion of incidence studies.

From table 1 it can be seen that the species of the genus *Acmaea* do not differ markedly from one another in inquiline population, and closely resemble *Lottia gigantea* in parasite species. The genera *Fissurella* and *Tegula* differ from each other and from the genus *Acmaea,* each having a fairly specific parasite population. All are characterized by being parasitized by *Scyphidia ubiquita,* a form that well may parasitize many gastropod species.

Without additional data regarding other Pacific coast areas, it is difficult to correlate the populations of *Fissurella* and *Tegula* from Big Rock and Flat Rock. Either might be the coastwise picture, or both may represent aberrant groups.

The description of *Eupoterion pernix* in limpets from the San Francisco bay area (MacLennan and Connell, 1931), and the brief mention of "urceolarids" found in limpets in the same area, in Kirby (1948) is indicative of a coastwise incidence of these forms.

The description of both urceolarids and scyphidians found by Cuenot (1891) in the European limpet, *Patella vulgata,* may indicate the existence of a worldwide peritrich fauna of limpets.

Seasons nor the sex or size of the host appeared to have any influence on the incidence of the parasites.

CROSS INFECTION STUDIES

Cross infections were attempted by pooling different host species in the same aquaria and allowing them to remain so mixed for a period of from several weeks to two months. In some instances naturally infected hosts were killed and the mantle cavity contents were added to the aquaria containing the forms to be infected.

It was found that in no instance did cross infection between turbans and limpets of either *Urceolaria* or *Trichodina* occur. In one instance *Tegula funebralis* was successfully infected with *Licnophora conklini* from *Fissurella volcano.* This is of some importance since a very low natural infection of *Licnophora conklini* was found in *Acmaea insessa,* indicating perhaps either a lesser degree of host specificity for *Licnophora* than in the case of *Urceolaria* and *Trichodina,* or perhaps a degree of inter-relationship between *Fissurella, Acmaea,* and *Tegula.*

MECHANISM OF INFECTION

Occasionally motile *Scyphidia* and *Urceolaria* were found in the aquaria. Although these occurred in low concentration and number, their presence indicates the probable method of infection of the hosts.

The motile forms of *Urceolaria* were found capable of survival for as long as four days when they were removed from the host and maintained in aerated syracuse dishes.

No information is available as to the method of infection by *Eupoterion pernix.*

TABLE 1.—*Parasite species*

Host species & no. sampled	*U. karyolobia*		*U. korschelti*		*T. tegula*		*S. ubiquita*		*E. perniæ*		*L. conklini*		Negative	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
A. pelta 110	95	86 ± 4	89	81 ± 4	0		77	70 ± 4	21	19 ± 4	0		7	6 ± 4
A. digitalis 193	180	93 ± 2	156	81 ± 3	0		97	50 ± 4	29	15 ± 2	0		2	1 ± 1
A. limatula 91	87	95 ± 2	54	59 ± 6	0		78	86 ± 4	10	11 ± 3	0		3	3 ± 2
A. scabra 62	31	50 ± 6	18	28 ± 6	0		24	39 ± 6	3	5 ± 3	0		20	32 ± 6
A. insessa 66	21	32 ± 6	16	25 ± 5	0		22	33 ± 6	0		2	3 ± 2	31	47 ± 6
A. fenestrata 71	61	86 ± 4	50	70 ± 5	0		50	70 ± 5	14	20 ± 4	0		4	5 ± 3
L. gigantia 36	36	100	26	70 ± 8	0		14	40 ± 8	6	17 ± 7	0		0	
F. volcano (1) 38	0		0		0		27	71 ± 8	0		0		11	29 ± 8
F. volcano (2) 34	0		0		0		22	65 ± 8	0		29	85 ± 7	1	3 ± 4
T. funebralis (1) 19	0		0		0		2	10 ± 7	0		0		17	90 ± 7
T. funebralis (2) 70	0		0		64	92 ± 4	66	94 ± 3	0		1*	1 ± 1	0	
T. ligulata 80	0		0		7	8 ± 3	74	92 ± 3	0		0		1	1 ± 1

(1) Collected at Big Rock Beach.
(2) Collected at Flat Rock Beach.
* May be an instance of cross infection.

Licnophora appears to infect its hosts by means of a free swimming stage, although no such stage was found in the aquaria (Stevens, 1904).

SUMMARY

The incidence, location and effect of the parasites on the host is given for the ten host species. No positive correlations were found between season influence, or sex and size of the host organism.

The ten host species surveyed are: *Acmaea pelta, A. digitalis, A. limatula, A. scabra, A. insessa, A. fenestrata fenestrata, Lottia gigantea, Fissurella volcano, Tegula funebralis* and *T. ligulata.*

The primary parasite fauna of the limpets and turbans studied belong to the ciliate order PERITRICHA. *Scyphidia ubiquita* is found to occur naturally in all ten host species.

The protozoa found primarily within the genus *Acmaea* are: *Urceolaria karyolobia, U. korschelti, Scyphidia ubiquita,* and *Eupoterion pernix. Acmaea insessa* differs from the other *Acmaea* examined in having no infection of *E. pernix,* and in having a low natural infection of *Lichnophora conklini.*

Members of the genus *Lottia* possess the same four inquilines as do the majority of the genus *Acmaea.*

The members of the genus *Fissurella* are found to differ from the members of the genera *Acmaea, Lottia* and *Tegula* in the absence of protozoa of the family URCEOLARIIDAE. A high natural incidence of *Licnophora conklini* is present. Two groups of *Fissurella* collected from two different sites differed markedly in their inquiline population.

The species of the genus *Tegula* harbor *Trichodina tegula* and *Scyphidia ubiquita* in high incidence.

Cross infection experiments between various host species are described. These experiments show that the members of the genera *Lottia, Acmaea* and *Fissurella* cannot be infected with *Trichodina tegula.* The members of the genus *Tegula* cannot be infected with *Urceolaria karyolobia, U. korschelti.* or *Eupoterion pernix.* Thus *Urceolaria karyolobia, U. korschelti, Eupoterion pernix* and *Trichodina tegula* exhibit some degree of host specificity.

The presence or absence of the six protozoan species with some other possible host species than the ten surveyed is reported.

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THREE NEW COPEPOD PARASITES OF THE RIBBON FISH FROM SOUTH INDIA

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Since 1905, when Wilson prepared a key for the over seventy-five known species of *Caligus,* more than forty-three species have been described. Yet of this number, those parasitic on Indian fishes appear limited to the few described by Bassett-Smith (1898a, b, c). There has been no intensive study of these forms, before or after this author. In the present paper, three new species of *Caligus,* parasitic on the ribbon fish *Trichiurus haumela* Forsker, one of the commonest fish in the markets of Madras, South India, are described.

I *Caligus cunicephalus* n. sp.

(Figs. 1–16)

Host and record: Two females and six males were collected from the Madras inshore plankton between December and February. Besides these a male and a female were found within the buccal cavity of a full grown *Trichiurus haumela.* All the forms were kept alive in the laboratory for over twenty-four hours and studied. Except the females with long egg strings, the others were swimming about actively. It is probable that this ectoparasite is capable of moving from the mouth-cavity to the skin and reaching another host if washed off the scaleless surface of the body.

Description of the female: 4.1 mm. long, exclusive of anal setae. 1.5 mm. broad without the flexible edge. Body whitish; median eyes, dark red. The carapace is less than half the total length, measuring 1.6 mm. and is about as long as broad. Anteriorly it is much narrower, making the carapace cuneiform. The thoracic area forms half the width of the truncated hind margin and extends behind the lateral lobes. A forwardly directed bristle occurs on either side of the median notch on the frontal plate. The furcal rami are as long as the base, divergent and clavate-tipped. The free segment is well marked, 0.4 mm. broad and half as long. It is constricted by a transverse groove into two rings. The genital segment is flask-shaped and has convex bulging sides. It is 1.3 mm. long and about 1.0 mm. across. The abdomen which is single-segmented and 0.6 mm. long, tapers behind. The anal laminae are long, narrow and bear three long and two short plumose setae. The egg strings measure 2 mm. long and about 0.16 mm. thick and hold 18–20 eggs uniserially.

Appendages: The proximal of the two joints of the first antenna bears twenty-seven plumose setae, while the distal segment is terminated by ten spines. The second antenna has a stout spine on its base and a small spine halfway on the terminal claw. The first maxilla is a long, sharp hook while the second maxilla, which has a cluster of three spines on its swollen base, is a long straight spine. The mouth tube is a broad-based cone of the usual structure. The mandibles bear 8–12 teeth of which the anterior five are larger. The first maxilliped bears two stout and curved spines as well as a third slender spine. The second maxilliped is unique in possessing two pairs of chitinous pegs on the basal joint to clutch the terminal hook when it is folded. There is also an accessory claw which further aids this appendage in its grip.

Swimming legs: The protopod of the first leg bears anteriorly on its distal margin a plumose seta, the vestigial endopod. The exopod has a spine on the outer end of the proximal joint, three posterior marginal plumose setae and four terminal spines on the distal article. The two-jointed protopod of the second leg is furnished with a plumose seta as well as a small spine. Of the three-jointed rami, the exopod carries three spines and eight plumose setae while the endopod bears nine plumose setae. The third leg is marked by a plumose seta on the protopod, five plumose setae and five spines on the exopod, and seven plumose setae on the two-articled endopod. The fourth leg, though uniramous, has at the tip of the long protopod, a small plumose seta representing the endopod. The two-jointed exopod is armed with five claw like spines of which the last is longer than the rest. The fifth leg is reduced to a papilla bearing two plumose spines, on the ventral side of the genital segment.

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Description of the male: 3.5 mm. long, 1.6 mm. across the carapace. Though the carapace is about the same size as in the female, it forms half the total length of the body because of the shorter genital segment, which is less than a third of the length of the carapace. The barrel-shaped genital segment being narrow makes the male appear more slender. The abdomen is relatively longer than that of the female. It is of two segments, the anterior being shorter than the posterior, and tapers to the slender anal laminae. In spite of these differences between the sexes, not uncommon in other species of *Caligus,* the appendages are almost identically alike.

Relationships: The present form resembles *C. schistonyx* Wilson (1905). But the form and proportion of the carapace, the genital and first adbominal segments, are different. The claws at the end of the first maxilliped and first leg, as well as the form of the second antenna and second maxilliped, distinguish the present species from Wilson's. Only four new species have been described from the Indian waters since 1905. Of these *C. sciaena, C. polycanthi, C. savala* were recorded by the author (1947, 1948b, 1948a) and *C. raniceps* by Heegard (1943) from a collection made by Capt. Sundevall from the Bay of Bengal, the host being unknown. The figures and description given by Heegard show that it is different from the present form. *C. savala,* collected from plankton as well as from the ribbon fish *Trichiurus savala* at Madras, though superficially similar to the present form differs in the number of setae on anal laminae, first antennae and second maxillae, in the form of the carapace, of the furcal rami, and of the abdomen, in the character of the second antenna and the two maxillipeds, and in the persistence of the sixth pair of legs.

The present form is therefore described as a new species, *Caligus cunicephalus,* and can be defined as follows: Carapace cuneiform less than half the total length in female and half the length in male. The free segment nearly half as broad as long. The genital segment of female is flask-shaped, nearly as long as carapace; in male it is barrel-shaped, and one third of the carapace in size. Abdomen single-segmented in female, two-jointed in male, the first joint being shorter than the second. Vestiges of the fifth leg are present and the furcal rami are clavate. The second maxilliped has two pairs of processes to clutch the terminal hook when folded.

Type host: Trichiurus haumela.

Location: Mouth Cavity.

Type locality: Madras, S. India.

Type specimens: Holotype (♀) and allotype (♂) will be lodged in the Indian Museum, Calcutta.

II *Caligus scabiei* n. sp.

(Figs. 17–30)

Host and record: A single female with spent egg strings was found attached to the skin of *Trichiurus haumela* in October 1947. It formed a thin transparent scab so firmly attached that it had to be pried up with tip of a fine needle. It was perfectly concave with the entire margin curved ventrally.

Size: 4.1 mm. long, exclusive of anal setae. 2.06 mm. broad across the cephalothorax.

External form: The carapace is nearly two thirds of the body in length, about 2.6 mm. It is broadly elliptical, the width three-fourths of the length. The region of the frontal plate and first antennae is conspicuous. Like the rest of the carapace it is convex above with the edges turned down, and hence the first antennae which are confined to the edges are invisible dorsally. A large median notch breaks the otherwise entire semi-circular frontal margin. The lateral sinuses on the posterior margin of the carapace, are deep and narrow, the median lobe which extends behind the lateral lobes, being five-eighths of the total width. The free segment is short and nearly four times as broad. The genital segment is quadrangular, the lateral sides are convex, the posterior margin concave and the hind corners are produced into rounded lobes. The ventral surface is folded into an apron-like plate. The abdomen is single-jointed, as broad as long. The anal laminae are broad and long. Each carries two short and three long setae terminally. The furca is ornate and distinctive. It consists of a short slender base carrying a circular plate whose posterior margin is deeply indented, forming two foliaceous rami.

Appendages: The first antenna is of two nearly equal segments flexed and pressed to the rim of the carapace where it constricts into the anterior frontal region. The proximal segment bears twenty-four setae of which only thirteen exhibit a plumose character, while the distal segment carries twelve spines terminally. A trowel-like spur marks the swollen base of the second antenna. The first maxilla is situated away from the margin, close to the second antenna and consists of a triangular plate with a stout long hook which bears a recurved prickle as well as two long setae. The mouth cone is broad with two wide lips. The mandibles, have falcate, finely toothed blades. The second maxilla has a blunt spur flattened into an oval blade. The first maxilliped has three hooks and a small plate, while the second maxilliped is weak and small,

out of all proportion to the size of the body. The first leg has a protopod bearing the two-jointed exopod carrying three marginal plumose setae and six distal spines. The two-jointed protopod of the second leg bears a plumose seta on its base. The three articles of the exopod bear three spines and nine plumose setae. The three-jointed exopod of third leg bears five spines and five plumose seta. The endopod is of two joints which carry five plumose setae. The fourth leg has a long protopod bearing the two-jointed exopod furnished with five spines. No vestiges of the fifth and sixth legs were seen.

Relationships: In spite of a general resemblance to *C. curtus* Muller, the rectangular genital segment with its width greater than the length and marked by two posterior lobes and a ventral flap, distinguishes the present form. Further, the form of the carapace, the details of the appendages and furca are different. Hence it is described as a new species *Caligus scabiei* and can be defined as follows: Carapace more than half the total length, broadly elliptical, strongly concave below, transparent and thin. Genital segment has two posterodorsal lobes and a ventral flap-like plate, abdomen one-jointed, furca plate-like ornate. The first maxillliped is long, slender, with three long spines and a plate, and the second maxilliped is weak.

Type host: Trichiurus haumela.

Location: Skin.

Type locality: Madras, S. India.

Type specimen: Holotype (♀) will be lodged in the Indian Museum, Calcutta.

III *Caligus longicervicis* n. sp.

(Figs. 31–46)

Host and record: Eleven adult females and one male were taken from inside the orobranchial cavity of nearly two dozen ribbon fishes (*Trichiurus haumela*) caught at Madras in December and January. Several chalimi were found attached to a cymothoan (*Irona* sp.) parasitic in the mouth of one of the fish. The chalimi, belonged to different sexes and of different ages. They were kept alive in the laboratory for a day and one moulted into an adult female. [The author is indebted to Mrs. F. G. Abraham of this laboratory, for the chalimi].

Description of female: 3.25 mm. long exclusive of anal setae and egg sacs. Dirty white with crimson reticulate markings. The semi-circular carapace which is strongly convex above, is 1.09 mm. long, is slightly broader than long, being 1.16 mm. at the truncated posterior margin. The frontal plate is conspicuous. It bears a forwardly directed bristle and large lunules with a small circular lobe outside each lunule. The lateral sinuses are very large and are directed outwards so that the lateral lobes appear narrow and curved inwards. The thoracic area is obtusely rounded behind but does not extend behind the lateral lobes. The free segment is unique in its length in a well-fixed specimen. It is more than a third of the length of the carapace and half as wide as long except where it gives articulation to the fourth leg. This gives the form a long-necked appearance. The genital segment is almost orbicular but for the narrow concave anterior margin and the more or less truncated posterior edge. In immature forms the genital segment appears rectangular with straight sides. It is slightly shorter than the carapace being 1 mm. long and slightly narrower than its own length. There are no posterolateral lobes but medially a short extension gives attachment to the abdomen. The abdomen is 0.44 mm. long, single-jointed, obovate in form. The anal laminae are long and narrow. Each bears three long, stout and three short, slender setae. The spermatophores are small, circular with umbonate thickenings. The eggs are large, nearly 0.3 mm. in diameter, a maximum of ten being contained in each sac. The furcal rami are thin, narrow and apparently separated. Under high power however, the triangular transparent base can be made out as in the figure.

Appendages: The first antenna has a basal segment with two dorsal and fourteen ventral plumose setae besides eight ventral spines. The club-shaped distal segment bears a dorsal, two ventral and eight terminal spines. The second antenna, mouth tube and mandibles are as in most species of the genus. The first maxilla is a long pointed hook with two setae on the base. The second maxilla consists of the usual robust hook as well as a stout seta on the base. The first maxilliped bears three long dactylose claws. The second maxilliped has a stout muscular basal joint as well as a robust recurved claw. The protopod of the first leg bears a small seta—the vestigial endopod. The two-jointed exopod carries three stout claw-like spines with lateral teeth, a long spine as well as three stout plumose setae. The two-articled protopod of the second leg has a plumose seta on its base. The three joints of the exopod carry three spines with serrate edges and eight plumose setae while those of the endopod are provided with nine plumose setae. The third leg is characterised by an exopod carrying five spines and four plumose setae, and a two-jointed endopod bearing seven plumose setae. The fourth leg has a seta at the tip of the protopod to represent the endopod. The two joints of the exopod bear five curved spines of increasing lengths, the terminal being the longest. No vestiges of the V and VI legs were seen.

Description of the male: 2.6 mm. long; 1.3 mm. broad across carapace. Except for differences in size, the appendages are similar. But the general form of the male is markedly different especially in a dorsal view. The IV segment shows annular constrictions. The genital segment is barrel-shaped, the width being slightly more than two-thirds the length. The length being only a little more than a third of the length of the carapace, the genital segment appears far smaller than in the female. The abdomen is two-jointed, and without the anal laminae; it is five-sixths of the genital segment. Owing to the smaller genital segment the carapace forms half the total body length.

Relationships: The present form, especially the male, resembles *C. haemulonis* Kröyer in several broad features but the poorly developed furca, the biramous second maxillae, the structure of the legs and the long free segment, mark the present form as distinct. In these respects, the present form differs from other species described since 1905 and is hence treated as a new species *Caligus longicervicis* and may be defined as follows: carapace deeply cup-shaped, semicircular with broad lateral sinuses; the free segment long, cylindrical, the genital segment of male barrel-shaped, of female stout, flask-shaped; the furca is poorly developed; the eggs large and few.

Type host: Trichiurus haumela.

Location: Branchial cavity.

Type specimen: The holotype (♀) as well as the allotype (♂) will be deposited in the Indian Museum, Calcutta and the paratypes in the University Laboratory.

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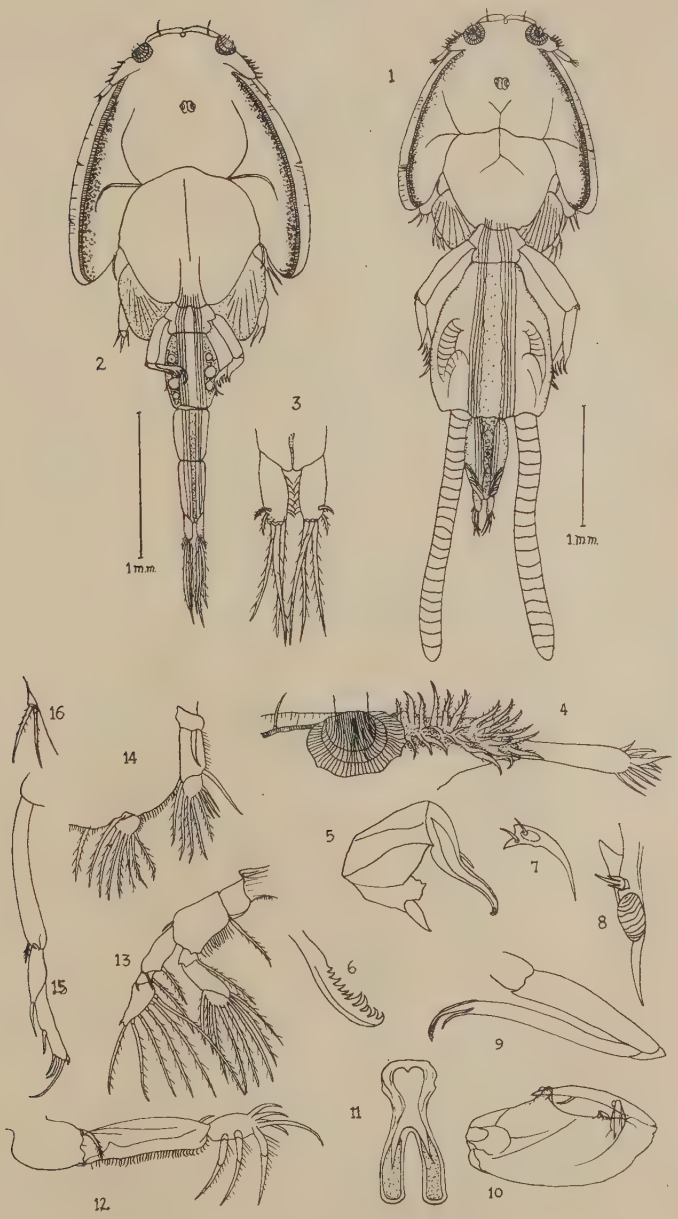
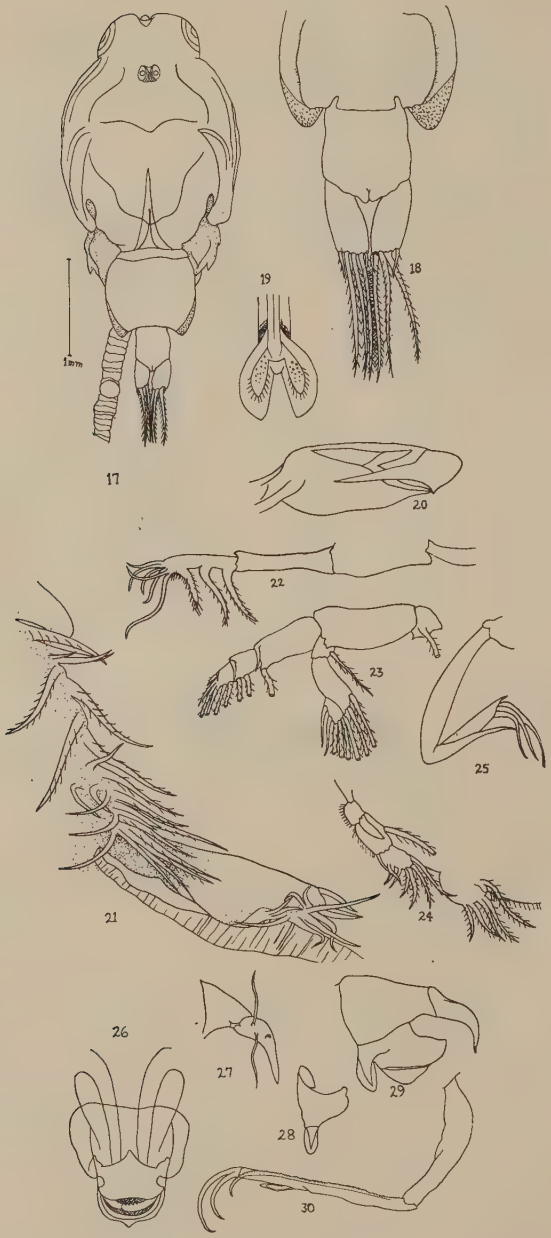
PLATE I



Caligus cunicephalus n. sp.

FIG. 1. Dorsal view of female. 2. Dorsal view of male. 3. Anal laminae. 4. First antennae and frontal plate. 5. Second antennae. 6. Mandible. 7. First maxilla. 8. Second maxilla. 9. First maxilliped. 10. Second maxilliped. 11. Furca. 12. First swimming leg. 13. Second leg. 14. Third leg. 15. Fourth leg. 16. Fifth leg vestige.

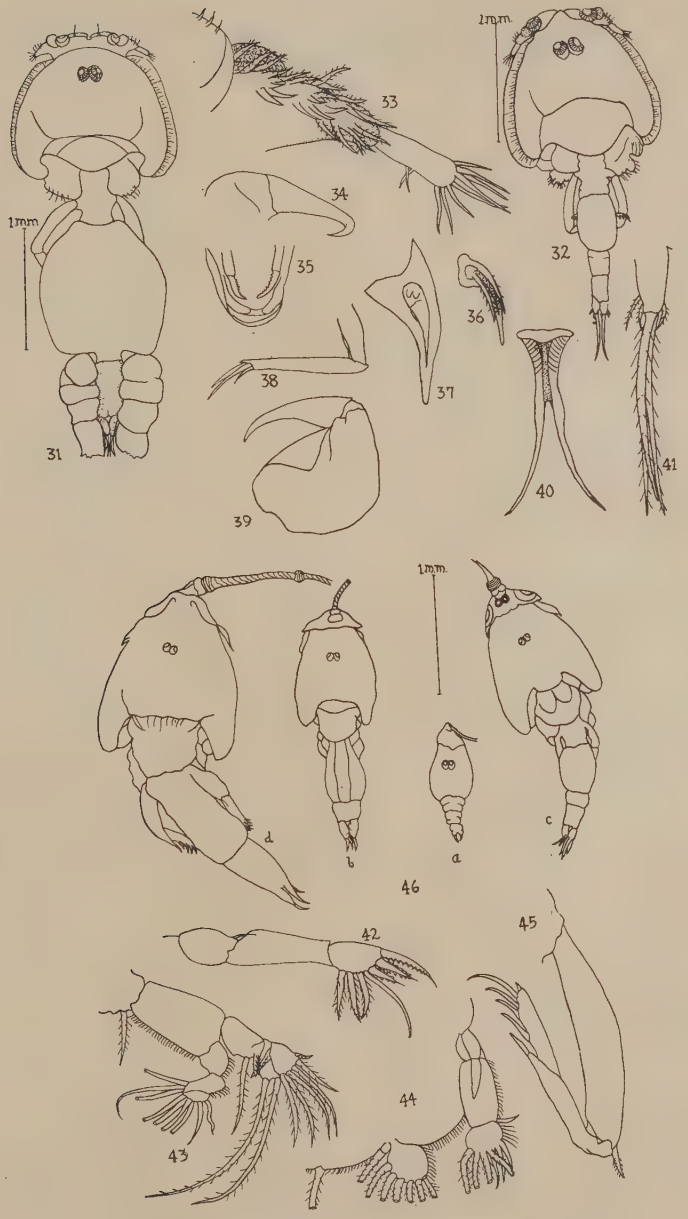
PLATE II



Caligus scabiei n. sp. female

FIG. 17. Dorsal view of female. 18. Ventral view of hind body. 19. Furca. 20. Second maxilliped. 21. First antenna. 22. First leg. 23. Second leg. 24. Third leg. 25. Fourth leg. 26. Mouth tube. 27. First maxilla. 28. Second maxilla. 29. Second antenna. 30. First maxilliped.

PLATE III



Caligus longicervicis n. sp.

FIG. 31. Dorsal view of female. 32. Dorsal view of male. 33. First antenna. 34. Second antenna. 35. Mouth tube. 36. First maxilla. 37. Second maxilla. 38. First maxilliped. 39. Second maxilliped. 40. Furca. 41. Ventral view of anal lamina. 42. First leg. 43. Second leg. 44. Third leg 45. Fourth leg. 46. Fourth chalimi, a, b, c, d.

BUCEPHALOPSIS KWEIYANGENSIS N. SP. FROM THE GIANT SALAMANDER, *MEGALOBATRACHUS JAPONICUS* TEMM. IN KWEICHOW, CHINA

H. J. Chu*

Gasterostomatous trematodes in their adult stage have until the present, as far as the writer is aware, been described only from marine- and fresh-water fishes, and no species has been reported from China. In 1947 the writer had an opportunity to collect numerous specimens from 8 giant salamanders in Kweiyang, capital of Kweichow Province. Examination showed that these specimens belong to the genus *Bucephalopsis* and represent a new species. The presence of these gasterostomatous trematodes in giant salamanders seems to be very common, as every one which was examined, was found to be infected.

In his recent publication Dawes (1946) lists altogether 19 species of *Bucephalopsis,* but points out that some species will probably fall after a review of the genus in the light of modern knowledge has been carried out. Some of the older descriptions are so incomplete and insufficient that they can hardly be used for species determination. One form *B. triglae* (Beneden, 1870), is furthermore known only by a figure.

The present species has been found different from species described from fresh-water fishes and also from those species described from marine fishes the description of which has been available to the writer. In view of their intermediate hosts it is in any case not likely that species reported from marine fishes will occur in the giant salamander, a strictly fresh-water animal. The life cycle of the present species has not yet been studied and nothing is known as to its intermediate host. It is possible that the same *Bucephalopsis* will occur in fresh-water fishes in Kweichow as they may live on somewhat similar food as the giant salamander. It will be of interest to study the occurence of *Bucephalopsis* species in fishes of different parts of China and also to compare them with those described from fresh-water fishes in India.

Bucephalopsis kweiyangensis n. sp.

Host: Adults in the giant salamander, *Megalobatrachus japonicus,* Temm.
Location: Large intestine.
Locality collected: Kweiyang, Kweichow Province, China.
Material: 70 specimens.
Type-specimen: Helminthological collection, Peiping Union Medical College, Peiping, China.
Description.

Size: 0.87–1.78 mm long, elongate-oval outline, broader anteriorly, maximum width 0.36–0.38 mm, colorless, except for the eggs.

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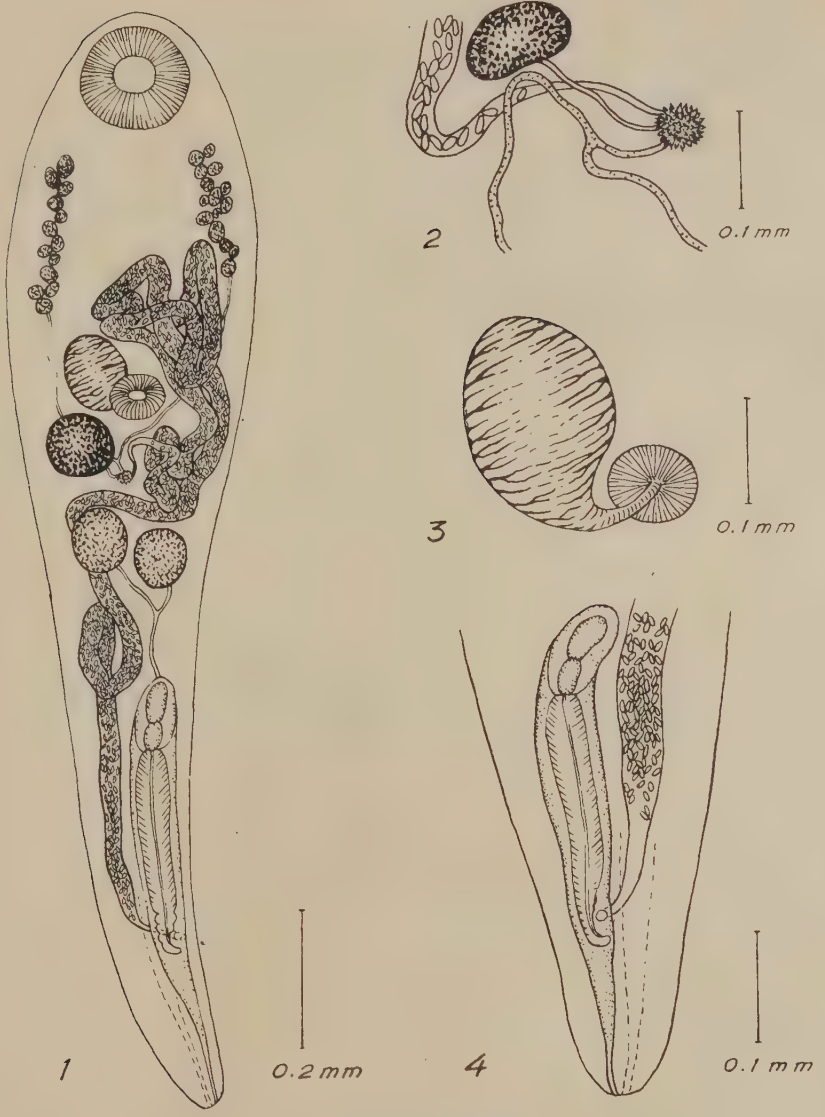

EXPLANATION OF PLATE

FIG. 1. *Bucephalopsis kweiyangensis* adult (composite drawing made from several specimens).

FIG. 2. Ovary and shell-gland with oviduct, common vitelline duct and proximal part of uterus.

FIG. 3. Sac-like intestine and curved oespohagus.

FIG. 4. Posterior extremity, showing genital pore and opening of excretory vesicle. In the cirrus pouch the bean-shaped vesicula seminalis, the short pars prostatica and the long cirrus are visible.

Cuticle: Provided with fine spines which are more numerous in the anterior part of the body than in the posterior region. Anterior sucker 165–174 microns in diameter.

Digestive system: Mouth situated about one-third of the body length from the anterior extremity. Pharynx with a strong muscular wall, 71–79 microns in diameter. Oesophagus comparatively long, 47 microns in length, curved; intestine, sac-like.

Reproductive system: Genital pore at the posterior extremity. Cirrus pouch slender and long, 553–616 × 63–79 microns, extending over more than one-third of the body length from the posterior extremity. It contains a bean-shaped vesicula seminalis, a short pars prostatica and a long cirrus without spines. Genital tongue small. Testes round to ovoid, 94–110 × 63–79 microns, close and almost parallel to each other and at about the same distance from the anterior and posterior extremities. Ovary globular to ovoid, 110–126 × 63–79 microns, anterior to the testes and close to the intestine, slightly on the right. Shell-gland antero-dorsal to the testes. Laurer's canal and receptaculum seminis could not be seen, but may be present. Vitellaria lateral in the anterior half of the body, consisting of 13–15 coarse follicles on each side. Uterus much convoluted with ascending and descending limbs, it opens into the genital sinus near the genital tongue. Eggs golden brown, 12–15 × 4–6 microns.

Excretory system: Excretory vesicle long and narrow, extending anteriorward beyond the cirrus pouch, the vesicle opens at the posterior extremity close to the genital pore.

This species may be distinguished by the following characters: location of the testes almost side by side; the long cirrus sac which, despite its length does not reach the testes; the long atrium which is more than one-third the length of the cirrus sac; and its occurrence in an amphibian host.

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THE CULTIVATION OF *PLASMODIUM LOPHURAE IN VITRO* IN CHICKEN ERYTHROCYTE SUSPENSIONS AND THE EFFECTS OF SOME CONSTITUENTS OF THE CULTURE MEDIUM UPON ITS GROWTH AND MULTIPLICATION

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Cultivation of the avian malaria parasite, *Plasmodium lophurae,* in suspensions of duck erythrocytes incubated in suitable media has been previously reported (Trager, 1947). Since adult chickens show a relative resistance to the parasite (Coggeshall, 1938; Terzian, 1941), and since it has been shown that their plasma when injected into young infected chicks depresses the parasitemia (Trager and McGhee, 1949), it seemed desirable to attempt the cultivation of *P. lophurae* in chicken erythrocyte suspensions and to study the effects on its development *in vitro* of plasma from chickens of different ages. Observations have also been made on the effects of other constituents of the culture medium.

MATERIALS AND METHODS

The 12A strain of *P. lophurae* (Coggeshall, 1938) which had been passed in young chicks every 5th or 6th day was used.

The basic culture medium was the same as that used by Anfinsen *et al* (1946) and Trager (1947) and is referred to as BGM. Concentrated stock solutions of the ingredients were prepared and stored in the refrigerator excepting the solution of ascorbic acid which was prepared and sterilized by filtration through a Selas 03 porcelain filter just prior to use.

The basic medium was modified in various ways in efforts to enhance parasite reproduction. Glutathione, which was prepared simultaneously with ascorbic acid and sterilized in the same manner, was incorporated into the completed medium. In certain experiments a medium was prepared with 1% bovine albumin and a portion of this medium used to replace the plasma of the normal blood. A commercial preparation of Fischer's tissue culture medium (Fischer et al, 1948) was also used.[1]

Culture media were distributed in 4.5 ml amounts into 50 ml Erlenmeyer flasks. Into each flask was pipetted 1.3 ml of normal blood secured from the neck veins of chickens of various ages, and 0.2 ml of parasitized blood from chicks which had been infected 4 days previously. Heparin was used as an anticoagulant except in experiment 6 in which the blood was defibrinated by shaking with glass beads. The flasks were fitted with inlet and outlet tubes and placed in an incubator. Cultures were subjected to moist 95% air with 5% CO_2, rocking, and a temperature of 39 to 40° C (Trager, 1947). Initial blood films were made just before incubation and additional films were made after 1 and 2 days of incubation. Parasites were counted in relation to 10,000 red blood cells and the numbers averaged for the flasks involved in the particular aspect of the experiment. Differential counts of 50

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[1] Obtained from Microbiological Associates, Inc., Flemington, N. J.

parasites were also made using the classification previously described (Trager, 1947).

Cultures were also prepared with red cells and plasma or fractions of plasma from chickens of various ages to determine any possible effects of age of the host upon the survival of parasites *in vitro*. In certain experiments normal red cells and plasma of chickens of various ages were added to the culture medium in the usual proportions. In experiment 9, equal amounts of adult plasma and culture medium were combined and 4.5 ml of the resulting mixture was dispensed into each flask. In two experiments the euglobulin fraction of normal adult plasma, precipitated from the plasma of several egg-laying hens by diluting it under aseptic conditions with 19 times its volume of distilled water, was added to the culture medium. In experiment 11 (preparation A) the euglobulin precipitate from 40 ml of plasma was dissolved in 15 ml of medium with the addition of 0.25 ml of a concentrated sodium chloride solution which gave a final volume of 20.25 ml. In experiment 12 a larger precipitate from 42 ml of plasma was dissolved in 7.6 ml of double strength calcium chloride-deficient basic medium to which was added 0.6 ml of distilled water and 0.4 ml of a concentrated calcium chloride solution bringing the volume to 16 ml of medium (preparation B). In both experiments 4.5 ml of the fortified media was pipetted into flasks, and cells and plasma of chickens of corresponding ages were added in the usual amount.

RESULTS

Details of the effects of various media upon the cultivation of *P. lophurae* in a chicken red cell suspension and for comparison the same parasite in suspensions of duck erythrocytes are given in Table 1. The morphological picture of cells and parasites and the increase in parasite numbers indicated the advisability of using the BGM medium in the presence of homologous plasma. The substitution of bovine albumin (fraction V of bovine plasma) for chicken plasma had a distinctly adverse influence upon the multiplication and morphology of the parasites. The addition of glutathione to the culture medium, although producing no significant changes in parasite numbers, resulted in fewer degenerate parasites. In Fischer's medium both the red cells and parasites were in worse condition than in the BGM medium. There was no discernible advantage attributable to the use of defibrinated blood in place of heparinized blood. Subcultures with a very low initial parasite density showed a higher rate of reproduction than the parent cultures.

Studies of the effects of plasma upon parasite reproduction were carried out using BGM medium with glutathione added (at a concentration of 100 mg %) in the presence of chicken plasma and red cells (Table 2). In sharp contrast to the effects *in vivo* of adult plasma and plasma fractions upon *P. lophurae* (Trager and McGhee, 1949) these materials had no significant effect on the multiplication of the parasite *in vitro*. Not only did cultures containing the euglobulin fraction of adult chicken plasma show no inhibition of growth, but in addition they presented after incubation for 2 days a picture of better preserved parasites and red cells than those seen in the control cultures.

DISCUSSION

In previous work (Trager, 1947) it was found that cultures of *P. lophurae* in duck red cell suspensions in certain instances tripled in numbers within 2 days. Al-

TABLE 1.—*The effect of various media upon the growth and multiplication of* P. lophurae *in vitro*
All flasks contained glutathione and chicken red cells and plasma unless otherwise indicated.

No. of experiment	No. of flasks	Medium	Parasites per 10,000 R. B. C. on days			Differential count of 50 parasites on days			Extent of multiplication
			0	1	2	0 Y–M–S*	1 Y–M–S	2 Y–M–S	
1	1, 2, 3, 4	BGM. Duck plasma and erythrocytes in place of chicken	564	1055	728	11–35– 4	20–23– 7	7–28–15	1.87
	5, 6, 7, 8	BGM	510	713	851	29–18– 3	9–27–14	10–24–16	1.67
2	1, 2, 3	BGM	553	819	716	21–26– 3	16–23–11	17–13–20	1.48
	4, 5, 6	BGM with 1% bovine plasma fraction V. No chicken plasma	738	654	531	38– 8– 4	3–34–13	7– 6–37	0.72
3	1, 2, 3	Subculture from Exp. 2 (1, 2, 3) in BGM	90	119	178	19–15–16	10–31– 9	24–10–16	1.98
4	1, 2	BGM without glutathione	371	386	423	29–15– 6	15–26– 9	11–22–17	1.17
	3, 4	BGM	391	431	612	31–15– 4	16–26– 8	24–15–11	1.56
5	4, 5, 6	BGM	855	1015	1088	39–11– 0	3–40– 7	33– 8– 9	1.27
	1, 2, 3	Fischer's	847	1083	997	38–12– 0	5–37– 8	22–16–12	1.28
6	1, 2, 3, 4	BGM + defibrinated blood	245	290	357	18–29– 3	4–24–22	6–26–18	1.46
	5, 6, 7, 8	BGM + heparinized blood	217	312	368	17–27– 6	4–23–23	9–24–17	1.69

* Y = small parasites without pigment; M, pigmented parasites with 1 to 3 nuclei; S, parasites with 4 or more nuclei.

TABLE 2.—*The effects of plasma from chickens of different ages upon the survival of* P. lophurae *in vitro*

No. of experiment	No. of flasks	Culture		Parasites per 10,000 R.B.C. on days			Differential count of 50 parasites on days			Extent of reproduction
		Age of plasma and cell donor	Medium	0	1	2	0 Y–M–S*	1 Y–M–S	2 Y–M–S	
7	1, 2, 3, 4	4 weeks	BGM	409	582	748	34–12– 4	7–35– 8	28–12–10	1.83
	5, 6, 7, 8	6 weeks	BGM	520	770	1002	31–13– 6	5–37– 8	27–13–10	1.94
8	1, 2, 3, 4	5 weeks	BGM	635	874	1001	31– 9–10	5–39– 6	27– 7–16	1.59
	5, 6, 7, 8	Adult hen	BGM	672	810	1199	36–12– 2	4–37– 9	28– 6–16	1.78
9	1, 2	5 weeks	BGM	483	683	700	10–34– 6	30– 8–12	15–31– 4	1.45
	3, 4	5 weeks	½ BGM + ½ plasma	607	620	725	12–37– 1	30– 9–11	7–42– 1	1.19
	5, 6	Adult hen	BGM	660	642	835	13–35– 2	27–13–10	12–35– 3	1.27
	7, 8	Adult hen	½ BGM + ½ plasma	774	868	1025	8–39– 3	36–10– 4	11–35– 4	1.32
10	1, 2, 3	5 weeks	BGM	383	438	567	36–10– 4	6–37– 7	24–11–15	1.48
	4, 5, 6	Adult hen	BGM	466	591	707	34–10– 6	26–15– 9	35– 9– 6	1.52
	7, 8, 9	Adult hen	BGM + euglobulin preparation A	588	668	866	35–12– 3	8–36– 6	31– 9–10	1.47
11	1, 2, 3	5 weeks	BGM	855	1015	1088	39–11– 0	3–40– 7	33– 8– 9	1.28
	4, 5, 6	Adult hen	BGM	909	1019	1455	38– 9– 3	5–33–12	35– 5–10	1.60
	7, 8, 9	Adult hen	BGM + euglobulin preparation B	869	1101	1145	35–13– 2	2–43– 5	25–11–14	1.32

* Explanation in Table 1.

though this rate was not achieved in this study, parasites in cultures containing duck red cells multiplied to a greater extent than in original cultures with chick red blood cell suspensions. This is in agreement with the greater pathogenicity of *P. lophurae* for ducklings than for chicks.

In cultures containing chicken cells the reproductive rate, although less than in cultures with duck cells, was nevertheless consistent. The delicate balance of conditions necessary for multiplication is indicated by the picture of poorly preserved parasites and red cells in Fischer's culture medium and in the BGM medium containing 1% bovine albumin but no chicken plasma. Euglobulin concentrates from hen plasma, which had been previously shown to reduce the parasitemia when they were injected into chicks infected with *P. lophurae* (Trager and McGhee, 1949), had no comparable effect in culture. Instead, the addition of these substances resulted in better preserved parasites and erythrocytes. It has been pointed out (Trager, 1947) that present culture methods for malaria parasites provide the optimum conditions for erythrocyte survival. Therefore, the better preservation of the erythrocytes in cultures fortified with the plasma fraction might have negated any adverse effects on the parasites. Finally it should be remembered that such factors as handling, incubation, and dilution might have affected the plasma fractions to the extent that their antimalarial properties were reduced or lost.

SUMMARY

The growth and multiplication of *P. lophurae* in suspensions of chicken red cells was favored by the use of BGM medium with the addition of glutathione. Adverse effects were noted following the substitution of bovine albumin for plasma and the use of Fischer's tissue culture medium. No effects, except possibly favorable ones, could be demonstrated by the use of adult chicken cells with adult plasma or a euglobulin fraction of adult plasma.

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THE SUBSTITUTION OF BACTERIA IN CULTURES OF *ENDAMOEBA HISTOLYTICA*

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The initiation of cultures of *Endamoeba histolytica* from microisolated cysts with various single species of bacteria was reported by Rees, Reardon, Jacobs, and Jones (1941) and Chinn, Jacobs, Reardon, and Rees (1942). In the course of these studies, 26 species of bacteria were tested for their ability to provide conditions in the Locke's-egg-rice medium suitable for the propagation of the protozoan. In column 1 of the table below are listed 13 species of bacteria with which cultures of

TABLE 1.—*Bacteria capable and incapable of supporting growth of* Endamoeba histolytica *as determined by different methods and investigators.*

From cysts*		From trophozoites†	
1 Growth	2 No Growth	3 Growth	4 No Growth
Organism ‡	*Escherichia coli*	*Bacillus niger*	*Bacillus megatherium*
Clostridium perfringens	*Aerobacter aerogenes*	*B. mesentericus*	*B. cereus*
Staph. aureus	*Alcaligenes faecalis*	*B. brevis*	*B. subtilis*
Strep. hemolyticus	*Staph. albus*	*Escherichia communior*	*Proteus vulgaris*
Strep. faecalis	*Proteus vulgaris*	*Vibrio comma*	*Escherichia acidi-lactici*
Strep. viridans	*Brucella suis*	*Neisseria catarrhalis*	*Pseudomonas aeruginosa*
Bacillus subtilis	*B. abortus*	.....	*Alcaligenes faecalis*
B. mesentericus	*Corynebacterium diphtheriae*	*Escherichia coli*‖	
Actinomyces muris	*Bacterium translucens*	*Klebsiella*¶ *pneumoniae*	
Aplanobacter stewartii	*B. striafaciens*	*Eberthella*¶ *typhosa*	
Bacterium coronafaciens	*Pseudomonas aeruginosa*	*Salmonella*¶ *paratyphi* A	
Serratia marcescens	*Salmonella schottmuelleri*	*S. paratyphi* B¶	
Neisseria catarrhalis	*Salmonella paratyphi*		
.....			
Streptobacillus sp.§			

* Organisms listed above the dotted line in column 1 and all in column 2 were reported by Chinn, Jacobs, Reardon, and Rees, 1942. National Institutes of Health strain number 101 of *E. histolytica* was used.

† Organisms listed above the dotted line in column 3 and all in column 4 were reported by Cleveland and Sanders, 1930. Various strains of *E. histolytica* were used.

‡ Originally reported as *Leptotrichia buccalis.*

§ Shaffer and Frye, 1948.

‖ Dobell, 1947, with many strains of *E. histolytica.* The author did not state whether the cultures originated from trophozoites or cysts.

¶ This report. NRS amoebae.

the amoeba were obtained. It is apparent from an inspection of this list that the bacteria are from widely separated taxonomic groups. In column 2 of the table are listed 13 species with which growth of *E. histolytica* was not obtained. Included among these are *Escherichia coli* and *Salmonella schottmuelleri,* which were found to support growth of amebae from microisolated cysts on one occasion but not on

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numerous retrials. This list shows that cultures of amoebae were not obtained with bacteria closely related to the species named in column 1.

Prior to the work reviewed above, *Endamoeba histolytica* had been established in cultures with single species of bacteria by Cleveland and Sanders in 1930. In the work of these authors, vegetative amoebae were removed from sterile amoebic liver abscesses in cats and cultivated in liver infusion agar medium with 6 species of bacteria, which are listed in column 3 of the table. Little or no growth of amoebae was obtained with 7 other species, named in column 4. Here again, the species of bacteria with which amoebic growth was obtained are from very different taxonomic groups, while cultures were not obtained with other closely related species.

To complete the data in the table, mention should be made of the cultivation of *E. histolytica* with *Escherichia coli* by Dobell (1947) and with a streptobacillus by Shaffer and Frye (1948).

Comparison of the results of these different investigators is not possible because various media were used and in some cases the cultures were initiated from cysts and in others from trophozoites. It was considered, because of the complicated factors operating in cultures of bacteria and amoebae, that even if excystation took place in some cultures inoculated with small numbers of cysts, growth of bacteria might either overun the metacystic amoebae or fail otherwise to support their development. On the other hand, a more favorable equilibrium might be established in cultures in which large numbers of amoebae were introduced with the bacteria. Consequently it was decided that results of interest might be obtained by testing the ability of various bacteria to support *Endamoeba histolytica* in the L.E.R. medium in cultures initiated from trophozoites rather than cysts.

Substitution experiments were therefore performed, in which cultures of amoebae growing with bacteria sensitive to penicillin were inoculated with penicillin-resistant bacteria and treated with the antibiotic to remove the original flora. The NRS strain of *Endamoeba histolytica* growing with *Streptococcus hemolyticus* was used. An attempt was made to substitute the following bacteria for the streptococcus: *Eberthella typhosa, Salmonella paratyphi* A, *S. paratyphi* B, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella dysenteriae, Salmonella enteritidis,* and *Proteus vulgaris* OX 19.

Inocula of each of these bacteria from 24-hour agar slant cultures were seeded by means of a bacteriologic loop into 3 newly transplanted NRS-streptococcus cultures each. Then 20 units of penicillin per ml. overlay were added to each of the tubes. At 48 hours, the cultures were transplanted into tubes of fresh L.E.R. medium with the same concentration of penicillin. At 96 hours, the original cultures and the transplants were examined for amoebae and all were found positive except those with *Pseudomonas aeruginosa.* Blood agar plates streaked at 24 hours showed a few streptococcus colonies; so in later transplants the dosage of penicillin was increased to 50 or 100 units per ml. overlay. After 2 more transplants at 3-day intervals, the cultures with *Salmonella enteritidis* no longer showed amoebae, and these cultures together with those of *P. aeruginosa* were discarded. All the other cultures showed good growth of the protozoan. Streak plates made on blood agar and Gram-stained smears showed streptococci in the cultures with *Shigella dysenteriae* and *Proteus vulgaris.* These cultures were also discarded. The remaining cultures of amoebae with *Klebsiella pneumoniae* and with the typhoid and

paratyphoid bacilli were carried on without penicillin after the fifth transplant. Purity tests done from time to time by means of blood agar plates and slants and Gram-stained smears failed to reveal the presence of streptococci. It is therefore believed that substitution of the Gram-negative bacilli for the streptococcus was accomplished in these cultures. Good growth of amoebae was obtained with these bacteria, and the cultures were maintained for over 6 months with transplants every 3 or 4 days.

It has thus been established that 3 additional species, including 2 strains of 1 of these, are capable singly of supporting growth of *Endamoeba histolytica* in the Locke's-egg-rice medium. These are included in the tabulation to make as complete a listing as possible of all the bacteria thus far tested. It is likely, considering the results reported here, that cultures of *Endamoeba histolytica* may be obtained with still other species of bacteria.

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THE EFFECT OF PARASITISM BY AN ENTONISCID ON THE SECONDARY SEX CHARACTERS OF *PAGURUS LONGICARPUS*

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The common hermit crab *Pagurus longicarpus* Say is parasitized at Woods Hole by two species of EPICARIDEA (a suborder of isopod crustaceans). A bopyrid, *Stegophryxus hyptius* Thompson, occurs attached to the abdomen and an entoniscid, *Paguritherium alatum* Reinhard, is found within the haemocoele. Simultaneous parasitism by both the bopyrid and the entoniscid is, however, of rare occurrence. *Stegophryxus* causes no external modifications of the host, neither does it effect any marked reduction of the gonads. *Paguritherium*, on the other hand, brings about complete castration of female hosts and more or less complete atrophy of the male gonads. The pleopods of infested males are unmodified, but those of infested female crabs, while normal in number, resemble the male type more than they do the type characteristic of normal adult females.

The effects of RHIZOCEPHALA and EPICARIDEA on the secondary sex characters of hermit crabs as reported in the literature fall into four categories:

I. *Male pleopods modified; female pleopods unaffected.* By RHIZOCEPHALA: *Pagurus cuanensis* (Nilsson-Cantell, 1926) and *Pagurus prideauxii*[1] (Potts, 1906; Baffoni, 1947). By EPICARIDEA: *Pagurus bernhardus* (Giard, 1887; Bonnier, 1900) and *Pagurus prideauxii* (Baffoni, 1947).

II. *Male and female pleopods both modified.* By RHIZOCEPHALA: *Pagurus meticulosus* (Potts, 1906) and *Pagurus samuelis* (Shiino, 1931).

III. *Male pleopods unaffected; female pleopods modified.* By RHIZOCEPHALA: *Pagurus bernhardus* (Giard, 1887; Guerin-Ganivet, 1911).

IV. *Male and female pleopods both unaffected.* By RHIZOCEPHALA: *Anapagurus chiroacanthus* (Nilsson-Cantell, 1926); *Pagurus pubescens* (Reinhard, 1942). By EPICARIDEA: *Pagurus longicarpus* (Thompson, 1901).

No case has been reported in which a female pagurid manifested alterations in its secondary sex characters under the influence of an epicaridean parasite and only a few instances, based on scarce material, where the presence of a rhizocephalan parasite affected the external characters of the female alone. It was therefore thought worth while to study the female pagurids infested with *Paguritherium* and analyze as completely as possible the modifications of the pleopods with respect to the size of the host, the amount of deviation from the normal and the precise nature of the changes to discover whether the alterations were a retention of the juvenile condition, a state of female intersexuality, or some other phenomenon.

MATERIALS AND METHODS

Both normal and parasitized crabs used in this study were collected by the senior author at the Marine Biological Laboratory, Woods Hole, Mass. during the summer

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[1] Females of *Pagurus prideauxii*, parasitized by *Peltogaster curvatus*, have pleopods less well developed than normal females, according to a more recent study by Baffoni (1948, Pubbl. Staz. Zool. Napoli, *21* (3) : 237–255). The parasitized males, however, exhibit typical feminization of secondary sex characters.

of 1946. Under a binocular dissecting microscope the hermit crabs were assorted into four groups: normal males, normal females, parasitized males and parasitized females. Although the presence of the internal parasite is readily recognizable without opening up the crab (Reinhard, 1945) all specimens used in compiling the data were dissected at the conclusion of the study to make sure they had been placed in the proper groups.

The infested pagurids, of which about 100 were available, were measured and assorted according to carapace length. The measurements were taken from the anteriormost point of the carapace projecting between the eyes to the indentation at its posterior margin.

In selecting the normal crabs to be examined, a sufficient number of specimens was picked out to correspond approximately with the number of parasitized crabs of the various size groups.

Since the pleopods were the primary object of study and in pagurids are the chief manifestations of external secondary sex differences these abdominal appendages were removed from each crab and mounted in glychrogel on slides in order that measurements and drawings might be made.

Each pleopod consists of a protopod and two rami known as the endopod and the exopod. The female abdomen carries four pleopods while the male has only three; the first (attached to the second abdominal segment in the female) is lacking in the male. In normal females the rami are more nearly equal in length than they are in normal males. It was necessary therefore to measure the length of the rami of each pleopod and obtain the ratio of the length of the endopod to that of the exopod in order to establish the average proportional length of the two rami per segment and per sex. With such data it would be possible to compare the parasitized crabs with the normal as was done by Shiino (1931).

More significant, however, would be a determination to discover which of the two rami were most modified through parasitism. This was done by calculating the relation of the length of each ramus to the carapace length, using the length of the carapace as an index of the general size of the crab.

Finally, the villosity on the pleopods was studied comparatively, since the tufts of hairs, particularly those on the outer margin of the endopod and protopod, differ with respect to size and number in the two sexes.

OBSERVATIONS

A. *Comparison of the Endopod/Exopod Ratio:—*

It will be seen from Table 1 that in the case of normal crabs the female has a greater endopod/exopod ratio than male crabs with respect to the pleopods of the third and fourth segments. There is no appreciable difference between normal males and females in the proportional length of the rami of the pleopod of the fifth segment. The averages of the combined classes are as follows:

	2nd seg.	3rd seg.	4th seg.	5th seg.
Norm. ♀♀	.81	.60	.47	.21
Norm. ♂♂		.28	.25	.23

It should be noted that the endopod/exopod ratio decreases in each crab proceeding from the anterior to the posterior appendage. Although both rami actually become smaller, the ratio is diminished largely through decrease in size of the endopod.

In the case of parasitized females the endopod/exopod ratio with respect to the pleopods of the third and fourth segments is considerably smaller than in normal female crabs: .45 and .34 for infested females as compared with .60 and .47, respectively, for normal females.

TABLE 1.—*Endopod/exopod ratios for the pleopods of normal and parasitized crabs.*

Carapace Length Class (mm.)	Number	Type	Pleopods			
			2nd Seg.	3rd Seg.	4th Seg.	5th Seg.
			FEMALES			
4.0–4.9	9	Norm.	.78	.52	.43	.23
	9	Par.	.73	.40	.32	.23
5.0–5.9	15	Norm.	.82	.62	.47	.21
	18	Par.	.74	.41	.31	.21
6.0–6.9	18	Norm.	.82	.63	.47	.20
	15	Par.	.86	.51	.34	.23
7.0–7.9	5	Norm.	.80	.64	.49	.18
	4	Par.	.88	.49	.39	.23
Total Average:	47	Norm.	.81	.60	.47	.21
	47	Par.	.80	.45	.34	.23
			MALES			
4.0–4.9	6	Norm.		.28	.25	.26
	5	Par.		.29	.28	.24
5.0–5.9	10	Norm.		.28	.28	.23
	14	Par.		.29	.26	.24
6.0–6.9	18	Norm.		.28	.24	.21
	22	Par.		.29	.25	.22
7.0–7.9	6	Norm.		.29	.25	.24
	5	Par.		.29	.26	.23
8.0–8.9	3	Norm.		.28	.23	.22
	0	Par.				
Total Average:	43	Norm.		.28	.25	.23
	46	Par.		.29	.26	.23

Parasitized male hosts showed no significant difference in endopod/exopod ratio compared with normal males.

B. *Comparison of the Endopod/Carapace Ratio:—*

The pleopods of the normal female retain somewhat the same endopod/carapace relationship throughout all the size classes. Therefore, as the crab grows so does the endopod increase proportionally in size (Table 2).

In the parasitized females the endopods of pleopods 1–3 are much smaller than the corresponding ones of normal crabs of the same size. This is shown by the diminished endopod/carapace ratio of infested females as compared with normal females (Table 2). The greatest reduction is exhibited by the second and third pleopods. No apparent change is produced in the endopod of the fourth pleopod and the data for this pleopod have therefore been omitted from the table.

With respect to parasitized males, little or no difference in endopod/carapace ratio was manifested when compared with normal males. The data for the males are therefore also omitted. It is interesting to note here that although normal male crabs were found with a carapace length as great as 9.0 mm., no infested male exceeded a length of 7.5 mm.

C. *Comparison of the Exopod/Carapace Ratio:—*

The exopod length in relation to the body size of the crab was calculated for all classes of normal and parasitized crabs, but because there were no appreciable differences the data are not presented here.

TABLE 2.—*Length of endopodite of pleopods in relation to carapace length in normal and parasitized female crabs.*

Carapace Length Class (mm.)	Number	Type	Endopod Length Range (mm.)	Av. E/C Ratio (per cent)
		PLEOPOD 1.		
4.0–4.9	9	Norm.	.45– .79	16
	9	Par.	.28– .77	12.5
5.0–5.9	15	Norm.	.72–1.0	16
	18	Par.	.48– .96	15
6.0–6.9	18	Norm.	1.1 –2.3	18
	15	Par.	.67–1.2	17
7.0–7.9	5	Norm.	.98–1.3	16
	4	Par.	1.1 –1.6	19
		PLEOPOD 2.		
4.0–4.9	9	Norm.	.82–1.1	21.5
	9	Par.	.41– .79	15
5.0–5.9	15	Norm.	1.0 –1.3	23
	18	Par.	.65–1.1	17
6.0–6.9	18	Norm.	1.2 –1.9	25.5
	15	Par.	.84–1.3	18.5
7.0–7.9	5	Norm.	1.6 –1.8	24
	4	Par.	1.3 –1.4	20
		PLEOPOD 3.		
4.0–4.9	9	Norm.	.55– .86	18.5
	8	Par.	.34– .60	11.5
5.0–5.9	15	Norm.	.82–1.0	17
	18	Par.	.48– .82	11.5
6.0–6.9	18	Norm.	.84–1.5	18
	15	Par.	.60– .98	12.5
7.0–7.9	5	Norm.	1.2 –1.4	18
	4	Par.	.91–1.0	14

D. *Comparison of the hairs present on the pleopods:—*

Only female crabs were studied thoroughly with respect to the tufts of hairs present on the pleopods since infested males, on preliminary examination, showed no modifications.

Within a given size class there was some variation in the number and length of the hairs. All the specimens from each size class were examined and the one that best represented the average condition was selcted as typical of the class. The appendages of the "average" crab of each size class were drawn to scale. From the many drawings that were prepared, three sets were finally selected to illustrate the following conditions: a) pleopods of a normal female; b) pleopods of a parasitized female showing slight modifications; c) pleopods of a parasitized female showing the greatest amount of modification. These drawings are reproduced in Figure 1.

With respect to the first pleopod, about 15 per cent of all parasitized females showed complete loss of hairs on the external margin of the endopod and 85 per cent showed partial loss. For the second pleopod the figures were approximately 25 per cent complete loss and 75 per cent partial loss; for the third pleopod, 98 per cent complete loss and 2 per cent partial loss.

The hairs on the outer margin of the protopod were at least partially absent from pleopods 1, 2 and 3 in all infested females. Twelve per cent showed complete loss of these hairs on the second pleopod and 24 per cent complete loss on the third pleopod.

It is evident again that pleopods 2 and 3 suffer the greatest modification. This is apparent in all size classes of infested females but more so in the smaller hosts of the 4 mm. class. The fourth pleopod showed no differences from the normal condition.

FIG. 1. Pleopods 1, 2 and 3 of female *Pagurus longicarpus* showing the effects of parasitism by *Paguritherium alatum*. Note the reduced villosity and the decrease in the proportional length of the endopod. A—Pleopods of normal female. B—Slight modification resulting from parasitism. C—Extreme modification. (Drawings by Rose Provasoli.)

DISCUSSION

In order to interpret correctly the observations reported in the previous section it is necessary to consider first the normal development of the pleopods.

In *Pagurus longicarpus,* according to Thompson (1903), the glaucothoë or last larval stage has a symmetrical abdomen with a pair of biramous appendages on each segment except the first. The rami are slightly developed and the appendage resembles that of a male. In the stage immediately following the glaucothoë the animal first seeks a shell. The abdomen is now asymmetrical and the pleopods of the right side have disappeared. The first pleopod of the left side remains small but the external rami of the other pleopods increase in size. At this time there are no external sex differences; both male and female crabs possess appendages with rudimentary internal rami. At the seventh or next moult the pleopod of the second segment is lost in males but retained in females. Development of the other pleopods into the characteristic female type requires several more moults for full completion. In *Pagurus bernhardus,* according to Pérez (1932) the immature females also have pleopods resembling those of the male.

It is with these facts in mind that we can now review the interpretations other investigators have placed on pleopod modifications in hermit crabs resulting from parasitism.

Giard (1888) concluded that "castration parasitaire" tends to produce in males the secondary sex characters of the opposite sex (e.g. *Pagurus* parasitized by *Athelges*), but might also arrest the development of the secondary sex characters in either sex (e.g., female pagurids infested with *Peltogaster*). Smith (1906) believed that only parasitized male hosts showed pronounced modifications (i.e. feminization) and never the reverse. The effects seen in the female he attributed to regressive changes assignable to a lowered metabolic condition. Potts (1906), in studying *Pagurus meticulosus* infested with *Peltogaster curavtus,* found sex reversal in the first three abdominal appendages of male crabs but the appendages of the parasitized females were unaffected except in the case of two specimens having pleopods that resembled the young rather than the adult females. He attributed the reduction of the endopod in these specimens to the possibility of early infestation.

Nilsson-Cantell (1926) in his studies on the effects of rhizocephalan parasites on hermit crabs contended that only the male hosts were modified. Shiino (1931) referred the effects to a tendency of the parasitized host towards the normal condition of the opposite sex.

In the young females of *P. longicarpus,* as we have seen, the internal rami of the pleopods are less developed than in the adult, but as the crab grows in size both rami increase proportionally with a greater increase, relatively speaking, for the endopod so that both rami become more nearly equal. In parasitized females, on the other hand, although the external rami increase in length during the growth of the crab quite as in normal specimens, the internal rami fail to develop normally. The effects of parasitism, then, is not to retard the development of the female pleopods as a whole, but to suppress that portion of the pleopod (the endopod) which is more precisely a secondary sex character. It should be recalled in this connection that the greater development of the endopods of the normal female pleopods as compared with normal males is of adaptive value in providing anchorage for the developing eggs. In both sexes the exopods of the abdominal appendages are

used in aerating the shell through reinforcing the currents which are primarily induced by the branchial outflow and movements of the body (Thompson, 1903).

The first three pleopods of the normal adult female, in order to serve their ovigerous purpose, are furnished on the external surface of the protopod and endopod with two tufts of particularly long and rigid hairs. It is to these hairs that the female attaches her eggs. Hairs are completely lacking on the protopod and endopod in the case of males and juvenile females. Absence of these hairs in parasitized females is therefore a modification of a special type of secondary sex character.

Giard (1888) noted similarly that in female *Pagurus bernhardus* parasitized by *Peltogaster* there was a more or less complete absence of the bouquets of ovigerous hairs on the protopod and endopod of the pleopods. The corresponding hairs on the pleopods of *Pagurus samuelis* were also noted by Shiino (1931) to be fewer in number and much shorter in parasitized females than in normal females.

The effects of *Paguritherium* on female *Pagurus longicarpus* cannot be explained as simply an arrest in development. If the general development were arrested one should find other juvenile features in the parasitized females in addition to undeveloped pleopods. The antennae and the opthalmic scale, for instance, which are different in the juvenile crab (Thompson, 1903), in infested females are exactly like those of normal adults. There is no need to explain the changes as a partial sex reversal, a leading towards the opposite sex, and the evidence does not support such a claim. Rather, the changes noted appear to be a suppression of the normal growth of the endopod, specifically of the second and third pleopods, and a suppression of the development of the ovigerous hairs. These are the chief *functional* secondary sex characters of the female.

Whether or not this failure of the endopods and ovigerous hairs to develop in parasitized specimens as they do in normal females is directly influenced by the destruction of the host gonads by the parasite is a question that cannot be answered categorically at present. *Paguritherium* does bring about complete castration of female hosts and this strongly suggests the possibility of a hormonal influence from the ovaries on the proper development of the endopods and ovigerous hairs, secondary sex characters which are directly concerned with care of the developing young.

SUMMARY

Paguritherium alatum Reinhard brings about modifications in the secondary sex characters of the female *Pagurus longicarpus* Say, but does not externally modify the male host. The effects of parasitism are the following:

1. The ratio of the lengths of the rami of the first three pleopods of infested females is significantly decreased. This is especially noted in the second and third pleopod. The fourth pleopod is unmodified.

2. In both normal and infested females the lengths of the rami increase as the crabs grow, but the effect of parasitism is to cause a pronounced retardation in the growth of the endopod and only a slight decrease in the growth of the exopod.

3. There is either a partial or complete loss of hairs on the external surface of the endopod and protopod.

4. These effects cannot be explained as a general arrest of development, nor as a reversal towards the opposite sex, but as a specific suppression of the normal development of the endopods and ovigerous hairs which are the chief functional secondary sex characters of the female.

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THE EXCRETORY SYSTEM IN TREMATODA. II. THE EXCRETORY SYSTEM OF *LOXOGENOIDES BICOLOR* (KRULL, 1933)[1]

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Recently the writer examined a small number (54) of bullfrogs, *Rana catesbeiana* Shaw, from Georgia and North Carolina. Two of these frogs harbored a fluke which was tentatively identified as *Loxogenes bicolor* Krull, 1933. One frog, harboring a single worm, came from a small lake near Mountain City in North Georgia while the second host, containing four flukes, was taken near Midville in South Central Georgia. In both instances the parasite was removed from rather large fibrous cysts which involved the terminal portion of the bile duct and one lobe of the pancreas.

In attempting to study the living material it was soon found that the deeply pigmented cuticle, the great thickness of the body and the numerous eggs in the uterus made this type of study impracticable. Thus, the worms were killed in hot (60° C.) Bouin's fixative, stained with Bullard's hematoxylin and either mounted whole or sectioned for study.

Sufficient details of the morphology could be made out from the whole mounts to confirm the tentative identification. However, only the posterior third of the body was clear enough (devoid of the uterus) for accurate observations on the contained anatomical parts. In this region the testes, the intestinal ceca, a portion of the excretory bladder and some of the finer tubules of the excretory system could be seen clearly. The tubules of the excretory system stood out prominently as deeply pigmented tubules; at least two groups of the capillary tubules on each side of the body could be traced accurately. The prominence of these pigmented tubules suggested the possibility of following the complete tubular system from the serially sectioned specimens. In the sectioned animals the tubules proved to be richly pigmented throughout, with the pigment beginning adjacent to the flame cell and ending with the entrance of the tubules into the bladder. The bladder did not show the presence of this pigment.

The excretory pore is dorsal, a short distance in front of the extreme caudal end of the body, and is usually situated at the bottom of a rather deep furrow which extends from the pore to the caudal extremity. The pore is guarded by a rather heavy sphincter. Internally the short neck opens out into the thick-walled bladder which passes forward to a level just posterior to the caudal boundary of the ovary. The thickness of the bladder wall is due in part to rather tall and large epithelial cells which line the bladder throughout, and in part to a well defined limiting membrane which supports the epithelium. Throughout most of the length of the bladder this organ is pressed in between the ceca so that its cavity is narrow laterally but deep dorso-ventrally. Near its anterior end the bladder flattens out somewhat and

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becomes expanded into a broad, thin organ which gives rise to the two short, blunt cornua. Each cornus receives a common collecting tubule at its recurved tip. Each common collecting tubule swings outward and upward from the bladder, in

The Excretory System of *Loxogenoides bicolor* (Krull, 1933)

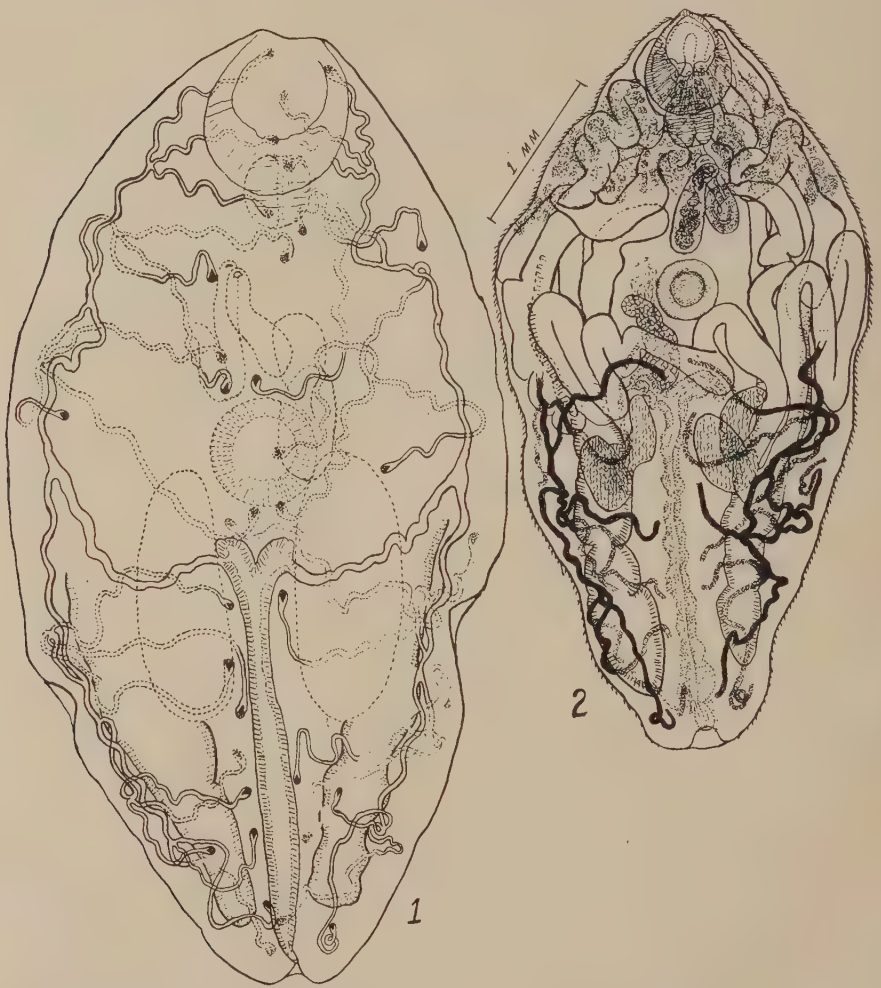

Fig. 1. Diagrammatic sketch of the excretory system of *Loxogenoides bicolor*. Outline of specimen drawn with the aid of the camera lucida. Ventral view. Drawn to the same scale as Fig. 2.

Fig. 2. Camera Lucida drawing of *Loxogenoides bicolor*, showing the position and arrangement of the internal anatomy and the heavily pigmented accessory and capillary tubules of the excretory system in the posterior half of the body. Ventral view of whole mount.

a slightly undulating course to a level approximating the caudal boundary of the acetabulum where, in the extra-cecal area, it is formed by the union of the two main collecting tubules on that side of the body. The anterior main collecting tubule on

each side ascends through a slightly undulating course in the extra-cecal and cecal areas to a level approximating the bifurcation of the ceca where in rather quick succession it is joined by the three accessory tubules on that side. The anterior-most accessory on each side is formed by the union of three capillary tubules which arise from flame cells located dorsally and laterally to the oral sucker. The second accessory on each side is joined by three capillaries which communicate with flame cells lying about the area of the pharynx and esophagus. The posterior-most accessory of each anterior main tubule passes posteriorly to near the level of the cephalic margin of the acetabulum before being formed by the fusion of three capillaries springing from flame cells lying in that area. The posterior main collecting tubule on each side descends through a slightly undulating course in the extra-cecal and cecal areas to near the level of the caudal boundary of the testes. Here each tubule is joined in rather quick succession by the three accessory tubules on that side. The posterior-most accessory is quite long, descending to near the ends of the ceca before being formed by the fusion of the three capillaries which arise from flame cells lying in the parenchyma about the ends of the ceca. The second accessory is short and is formed by capillaries arising from flame cells located in the area about the testes. The anterior-most accessory of each posterior main tubule receives three capillaries which originate from flame cells located just caudal to the acetabulum. Thus, the excretory system of *Loxogenoides bicolor* consists of twelve groups of flame cells, with three flame cells to each group. The pattern, then, is represented by the formula $2[(3+3+3)+(3+3+3)]$.

DISCUSSION

Believing his new species (*Loxogenes bicolor*) to be congeneric with *Loxogenes arcanum* (Nickerson, 1900), Krull (1933) stated: "*Loxogenes bicolor* is superficially very different from *L. arcanum* (Nickerson, 1900) Stafford, 1905, on account of its larger size, pigmented cuticula, and position of the vitellaria and genital pore, but the basic characters correspond to those of the genus; these differences, however, necessitate a slight emendation of the generic diagnosis, ..." It is apparent from the generic diagnosis as given by Krull "these differences" refer not only to the enumerated differences but equally as well to the longer ceca, the Y-shaped excretory bladder (as opposed to the V-shaped one) and to the differently located gonads. In believing the two forms to be congeneric, Krull not only accepted the family LECITHODENDRIIDAE as the proper allocation of the form but called specific attention to this fact.

Kaw (1945) reviewed the genus *Loxogenes,* and on the basis of published morphological studies recognized the differences between *L. arcanum* and *L. bicolor* as being of generic importance. Kaw created the genus *Loxogenoides* for the reception of *L. bicolor.* The writer is in complete agreement with this action.

The allocation of the genus *Loxogenoides* to the family LECITHODENDRIIDAE poses the question: What is the family LECITHODENDRIIDAE? Looss (1896) erected the subfamily LECITHODENDRIINAE for the reception of a group of morphologically similar worms. Later, however, he (1899) reconsidered the group and transferred the type genus, *Lecithodendrium,* to the subfamily BRACHYCOELIINAE. Lühe (1900) accepted this latter allocation of the type genus, although Odhner (1910) resurrected the subfamily LECITHODENDRIINAE and proposed the family LECITHO-

DENDRIIDAE for its reception. Cort (1919a, 1919b) discussed the relationship of his *Cercaria polyadena,* on the one hand, and *Margeana californiensis* (which he assigned to the subfamily BRACHYCOELIINAE), on the other, to those forms of the families PLAGIORCHIIDAE and LECITHODENDRIIDAE about which information relative to the excretory system was available. Cort concluded that the similarity in number of flame cells per group and the number of groups of flame cells in these forms outweighed the dissimilarities in the excretory bladder and general morphology in indicating their close relationship. Faust (1929, 1932) concluded from a study on the excretory system in the DIGENEA that the family LECITHODENDRIIDAE was more closely related to the family HETEROPHYIDAE than to the other families of the TREMATODA, and included it along with other families in his superfamily HETEROPHYOIDEA. Srivastava (1934) and Mehra (1935) treated the family LECITHODENDRIIDAE as a separate and distinct family group. More recently McMullen (1937) used the presence of a "Xiphidiocercaria" in the life cycle of the LECITHODENDRIIDAE as the principal criterion for relating the family to other families in the superfamily PLAGIORCHIOIDEA Dollfus.

Okabe (1937) recorded the most complete developmental cycle yet to be reported for a species currently assigned to the family LECITHODENDRIIDAE. This worker observed a "Virgula" type "Xiphidiocercaria" developing in small, ovoid sporocysts in the Japanese snail, *Bulinus kiushuensis.* These cercariae penetrated into and ultimately encysted in various species of dragon-fly nymphs. When these metacercariae were fed to frogs, young, sexually mature *Loxogenes liberum* Senoo, 1907, were recovered. Azim (1936), working with a "Virgula" type "Xiphidiocercaria" in Egypt, and Seitner (1945), working with a cercaria of the same type in this country, concluded from epidemiological evidence that they were dealing with the larval stage of *Lecithodendrium pyramidum* and *Loxogenes bicolor,* respectively. McMullen (1936), Yamaguti (1937) and Crawford (1938) were successful in obtaining sexually mature individuals of *Mosesia chordeilesia, Loxogenes liberum* and *L. arcanum,* respectively, following experiments in which they fed metacercariae from nymphs and naiads of dragon- and may-flies to suspected definitive hosts. It is unfortunate that these latter investigators failed to find or to produce experimentally the cercarial stage of these species, for information on the type cercaria associated with each of these forms would have added materially to our knowledge of the family group. On the basis of epidemiological evidence and morphological similarities between metacercaria and adult, Brown (1933) was able to conclude that the form with which he worked was the encysted form of *Lecithodendrium chilostomum,* a common parasite of the bat in England. Some of the forms with which Brown worked were young and immature enough to be considered recently penetrated cercariae. As a result, Brown postulated the morphology of the cercaria giving rise to the metacercariae with which he dealt. This postulated cercaria failed to possess the "Virgula" organ. The absence of this organ in Brown's material might now be expected since both Azim and Okabe noted the very rapid disappearance of the organ following entry of the cercaria into the arthropod host.

Some information is available on the excretory system for certain species of the family LECITHODENDRIIDAE. Looss (1894) indicated the flame cell pattern to be $2[(3+3+3)+(3+3+3)]$ for *Pleurogenes medians, P. claviger* and *Prosotocus confusus.* The same writer (1896) found the pattern to be $2[(3)+(3+3)]$ for

Lecithodendrium chefresianum, while for *Anchitrema sanguineum* it was given as $2[(2+3+3)+(3+3+2)]$. Faust (1919) found the pattern to be $2[(3+3)+(3+3)]$ for *Acanthatrium nycteridis*. The metacercaria which Brown (1933) considered to be a developmental stage of *Lecithodendrium chilostomum* showed a flame cell pattern of $2[(2+2+2)+(2+2+2)]$. An identical pattern was determined for the "Virgula" type cercaria which Seitner (1945) considered to be the larva of *Loxogenes bicolor*. The present paper reports a flame cell pattern of $2[(3+3+3)+(3+3+3)]$ for *Loxogenoides bicolor*.

From the information available at the present time relative to the developmental cycle and excretory system of species currently assigned to the family LECITHODENDRIIDAE it would appear an impossible task to offer a sound answer to the question posed above. It is clearly evident, however, that detailed morphological (to include all organ systems in the trematode body) and developmental studies must go hand-in-hand if a sound basis is to be established for the construction of lasting family groups. The confusion currently existing for the assigned species of the lecithodendriid family, then, should serve only to emphasize the urgent need for further intensive study on this group.

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THE GERMINAL MASS IN THE REDIAE OF *TRIGANODISTOMUM MUTABILE* (CORT) (TREMATODA: LISSORCHIIDAE)*

W. W. Cort, D. J. Ameel, and Anne Van der Woude

INTRODUCTION

A tailless cercaria developing in sausage-shaped rediae was described by Cort (1918) as *Cercariaeum mutabile* from *Helisoma campanulatum smithii* (Baker) from Douglas Lake, Michigan. This form has a very characteristic excretory system with a thick-walled club-shaped bladder and main excretory tubes which pass almost to the anterior end and loop backward before they branch into secondary tubules. There are eight groups of four flame cells each on each side. Other adult characters are very well developed, no stylet is present, but there are two groups of 6 penetration glands opening at the anterior tip dorsad of the oral sucker (Wallace, 1941, fig. 14). Sewell (1922) made this species the type of a larval group which he called the "Mutabile" group. Nine different species of larval trematodes have been described that can be assigned to this group from Europe, India, the United States, China, and Japan. Although some of these forms are poorly described they seem to form a natural group, the adults of which would be expected to be closely related, probably belonging to the same natural family or at least superfamily.

The life cycle of *C. mutabile* was worked out by Wallace (1941). He found that when the cercaria of this species was eaten by *Chaetogaster* or *Planaria* it penetrated the wall of the digestive tract and became encysted. Adults were obtained in both experimental and natural infections in the lake chub-sucker, *Erimyzon sucetta kennerlii Giard;* since they were considered to belong to the genus *Triganodistomum* Simer, 1919, the name of this species became *Triganodistomum mutabile* (Cort, 1918).

Wallace (1941) called attention to the similarity of the adult of *T. mutabile* to *Lissorchis fairporti* Magath, 1917 and tentatively placed it in the family Lissorchiidae Poche, 1925. He based this conclusion on a comparison of his adults of *T. mutabile* with adult specimens of *L. fairporti* sent him by Magath. In fact, this similarity is so close that the placing of these two species in the same family does not seem to be open to question (cf. Magath, 1917, pl. 1, fig. 1 and Wallace, 1941, pl. 1, fig. 6). However, the larval stages which Magath related to *L. fairporti* are entirely different from those of *T. mutabile*. The cercaria is a typical xiphidiocercaria that develops in sac-like daughter sporocysts. It has a typical Y-shaped excretory bladder with anterior and posterior collecting tubes opening into the tip of each horn. In fact, it is very similar to the cercariae that McMullen (1937) found to develop into adults of the genus *Plagiorchis*. We cannot agree with Wallace's conclusion that the cercaria of *T. mutabile* resembles Magath's cercaria except for the absence of a tail and a stylet. The excretory systems of these two forms seem to us to be fundamentally different, and the fact that one develops in rediae and the

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other in daughter sporocysts would in itself indicate that they do not belong in the same natural family. In fact, it would seem to us impossible that two such closely related trematode species as *T. mutabile* and *L. fairporti* could have larval stages as different as those described.

Faced with this dilemma we reviewed again the evidence that Magath presented for connecting his xiphidiocercaria from *Planorbis trivolvis* with the adults of *Lissorchis fairporti* which he found in the buffalo fish. It is of the type that has led to so many incorrect conclusions in the work that has been done over the years on the life cycles of the digenetic trematodes. The longest that he carried any of his infection experiments was only 8 days, and the worms that he got from them showed little if any development in structure beyond the cercaria body (cf. pl. I, figs. 3 and 6). He stated that "immature forms obtained from the experimentally infected fish are exactly like those found in natural infections." However, he did not give detailed descriptions or figures demonstrating these likenesses. He also concluded that the excretory system of the immature stage he obtained in his infection experiments was identical with that of the adult of *L. fairporti.* A careful comparison of figures 6 and 2 of plate I of Magath's paper shows in the first place that the excretory systems in both cases were very incompletely worked out. It looks to us as if the system in the adult of *L. fairporti* (fig. 2) resembles more closely that of *T. mutabile* than that of the xiphidiocercaria (fig. 3) and the stage that developed from it in the experimental infections (fig. 6). The turning back of the main collecting tube on each side of the pharynx in Magath's figure 2 is like that of *T. mutabile.* Also, the drawing of posterior collecting tubes on each side as connecting with the anterior collecting tubes near where they enter the club-shaped bladder would be a mistake very easy to make when we consider the complications of an excretory system of this type. Therefore, we are forced to conclude that the evidence presented by Magath that the xiphidiocercaria which he found in *H. trivolvis* belongs to *L. fairporti* is entirely inadequate, and also that this relationship is extremely improbable because it would establish for two adults obviously closely related, larval stages that are very different in so many respects.

MATERIALS AND METHODS

In our examinations of species of *Helisoma* in the Douglas Lake region during the summers of 1947 and 1948, we not infrequently found the larval stages of *T. mutabile* in *H. campanulatum smithii* (Baker) from Douglas Lake and in *H. antrosum percarinatum* (Walker) from Burt Lake. All of the infections of this species except three contained only daughter rediae and cercariae. In these three snails mother rediae were present, a stage which had apparently not been previously observed for this species. In a specimen of *H. antrosum percarinatum* from Burt Lake we found early in the summer of 1947 three very small immature rediae which from their structure (Fig. 1) obviously belonged to this species. We considered them to be very immature mother rediae since no other stages were found in this snail. It seems probable that a mother sporocyst must also have been present, but it was not found. Early in the summer of 1948 in one of our collections of *H. campanulatum smithii* from Douglas Lake we found one old mother redia in a mature infection. It was about 3 mm long and 0.6 mm wide and contained 7 mature daughter rediae which filled all the space in its body cavity. These daughters were

exactly like the others in the same infection and contained fully developed cercariae. We interpreted it to be an old persistent mother redia in which the last of its daughter redial progeny had failed to escape and had developed precociously while still in the mother. We also found mother rediae in a juvenile of *H. campanulatum smithii* from Douglas Lake collected in August, 1948. They were rather abnormal in appearance but contained large germinal masses and a few developing embryos (Figs. 2 and 3). Most of our other infections with this species contained only mature or old daughter rediae, and in only a few cases were immature daughter rediae present (Figs. 4 and 5).

As in our other studies on germinal material all observations were made on living material.

The Germinal Mass in the Rediae of *T. Mutabile*

The germinal masses of *T. mutabile* are alike in both mother and daughter rediae. They are characteristically very large and somewhat flattened (Figs. 5, 6 and 8) and are very strongly attached at the posterior end of the body cavity. They contain numerous components both unicellular and multicellular. The embryos (multicellular components) attain a larger size while still attached than do those in the germinal masses of most other trematode rediae. These germinal masses are very discrete structures and resist considerable mauling from the activity of the cercariae. In only a few cases were they flattened by the pressure of the cercariae as in one old redia that was examined (Fig. 9). One abnormal case was seen (Fig. 10) in another very old redia in which the germinal mass was broken up into parts and was on one side a little in front of the posterior end. The large size and complexity of the germinal mass in the rediae of *T. mutabile* is shown clearly in the figures and was found to be a general characteristic of this species. The length of the germinal mass shown in figure 6, is almost one-third that of the whole redia. The germinal mass in figure 7 measures 0.084 by 0.064 mm, and in one very immature daughter redia which is not figured, which was 0.18 by 0.085 mm, the germinal mass measured 0.06 by 0.04 mm.

It is evident that the germinal masses of the mother and daughter rediae of *T. mutabile* have the potentiality of producing large numbers of cercariae, since they contain at all stages considerable numbers of unicellular components and persist and continue to produce embryos in mature and old rediae. It should be noted, however, that not infrequently they were not found in very old daughter rediae indicating that they had been used up completely in the production of embryos.

Size and Number of Rediae

Mature and old daughter rediae of *T. mutabile* vary greatly in size, depending at least to some extent on the number that have developed in the digestive gland of the infected snail. In some cases rediae containing cercariae that were ready to escape were only from 0.29 to 0.30 mm in length by 0.09 to 0.13 mm in width. Such rediae sometimes contained as many as 4 to 7 mature cercariae besides 5 to 6 developing embryos. In one old infection, however, the rediae averaged about 0.30 by 0.15 mm and usually contained only one fully developed cercaria with about 6 embryos in various stages of development. Many infections had rediae from about 0.50 by 0.70 mm in length, which usually contained about 10 to 12 embryos, 2 or 3 of which were fully developed cercariae. A few larger rediae were measured; one

0.90 by 0.30 mm contained a total of 19 embryos; one 1.15 mm long had 3 well developed cercariae and 4 developing embryos; and the largest, 1.27 mm by 0.15 mm, had 10 well developed cercariae and 4 small embryos (Fig. 10). It is, therefore characteristic of the daughter rediae of *T. mutabile* that they contain only a small number of large fully developed cercariae, and only a few embryos in different stages of development. The large size of the cercariae as compared with the size of the rediae is very striking in this species. Wallace (1941) gave the range in size of six cercariae killed in hot formalin from 0.261 to 0.286 mm in length and from 0.065 to 0.078 mm in width.

Counts were made of the daughter rediae in 18 infected adult snails, which were about 10 to 12 mm in shell diameter. They varied from 273 to 3119 with an average of 1315; 15 of the counts had a range from 873 to 1913. These rediae and the considerable number of cercariae that were always found outside the rediae fill rather completely the digestive gland of the snail hosts.

In spite of the rather small number of mature cercariae and developing embryos in each redia we have the impression that a rather large number of cercariae escape from an infected snail each day although no actual counts have been made. This would be possible because of the large number of rediae in each infection which over the total reproductive period of the infection would each have to produce only a moderate number of cercariae to result in the production of a considerable number of individuals. It appears evident, therefore, that in this species the large size and precocious development of the cercaria has been achieved without very much reduction in the numbers produced in a single infection.

DISCUSSION

One of the most striking things about the germinal masses in the rediae of *T. mutabile* is their large size. Only in a very few instances are the germinal masses in the representatives of the Fasciolatoidea anywhere nearly as large as those of this species (Cort, Ameel, and Van der Woude, 1948). In actual size the germinal masses in *Halipegus eccentricus* are larger and more complex (Ameel, Cort, and Van der Woude, 1949, figs. 9 and 17), but they are not as prominent as those of *T. mutabile* because the mature germinal sacs of *Halipegus* are so much larger. In some of the daughter sporocysts of plagiorchiids the floating germinal masses are as large and complex as those of *T. mutabile*, and in some of the immature daughter sporocysts they occupy as large a proportion of the germinal sacs (Cort and Ameel, 1944, figs. 6, 7, and 8).

The similarity of the germinal masses in the rediae of *T. mutabile* to those in the daughter sporocysts of plagiorchiids is very striking (cf. Cort and Ameel, 1944, figs. 5, 6 and 7 with figs. 3, 4, 6, 7 and 8 of this paper). The only significant difference is that in the daughter sporocysts of the plagiorchiids the germinal masses are free in the body cavity while in the rediae of *T. mutabile* they are attached firmly at its posterior end. This likeness may be taken as additional evidence of the fundamental homology of plagiorchiid daughter sporocysts which have a single floating germinal mass, with the rediae in the groups that have a complex germinal mass attached at the posterior end of the body cavity. It also suggests how the floating germinal masses in the daughter sporocysts may have evolved. Investigations of other groups may well show cases in which daughter sporocysts will have germinal

masses attached at the posterior end of the body cavity. In fact, the descriptions of the germinal development in daughter sporocysts of *Dicrocoelium dendriticum* by Mattes (1936) and Neuhaus (1936, Fig. 12) and that by Denton (1945) for *Brachylecithum americanum* at least suggest that in these dicrocoeliids the daughter sporocysts have complex germinal masses attached at the posterior end of their body cavities. Also, the striking similarity of the germinal masses of *T. mutabile* with those of the plagiorchiids may be considered as adding to the evidence presented by Wallace (1941) that the LISSORCHIIDAE are related to the PLAGIORCHIOIDEA.

SUMMARY

The germinal masses in the mother and daughter rediae of *Triganodistomum mutabile* were found to be unusually large and complex as compared with those in the other groups that have been studied. In this species the cercariae, which are without a tail and which have adult structures unusually well developed, are very large in proportion to the size of the daughter rediae, and only a comparatively few cercariae and developing embryos are present in these rediae. In spite of this, the large number of daughter rediae in an infected snail and the persistence of the large germinal masses provide for the production of large numbers of individuals. The large germinal masses attached at the posterior end of the rediae of this species resemble the floating germinal masses of the daughter sporocysts of the PLAGIORCHIOIDEA, and add to the evidence that the LISSORCHIIDAE are related to this group.

The adult of *T. mutabile* resembles so closely that of *Lissorchis fairporti* Magath, 1917, that the two forms appear to be closely related. The fact that the larval stages described for these two species are so entirely different, suggests that the xiphidiocercaria that Magath assigned to *L. fairporti* is not really the larval stage of this species.

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Description of Figures

Germinal masses in mother and daughter rediae of *Triangodistomum mutabile.*

Fig. 1. Immature mother redia, 0.33 mm in length, from *H. antrosum percarinatum.*

Fig. 2. Mother redia, 0.255 by 0.180 mm, from H. *campanulatum smithii.*

Fig. 3. Mother redia, 0.30 by 0.21 mm, from *H. campanulatum smithii.*

Fig. 4. Very small immature daughter redia, 0.11 by 0.06 mm, from *H. campanulatum smithii.*

Fig. 5. Very small immature daughter redia, 0.135 by 0.72 mm, from *H. campanulatum smithii.*

Fig. 6. Posterior end of large immature daughter redia, 0.66 mm in length, from *H. campanulatum smithii.*

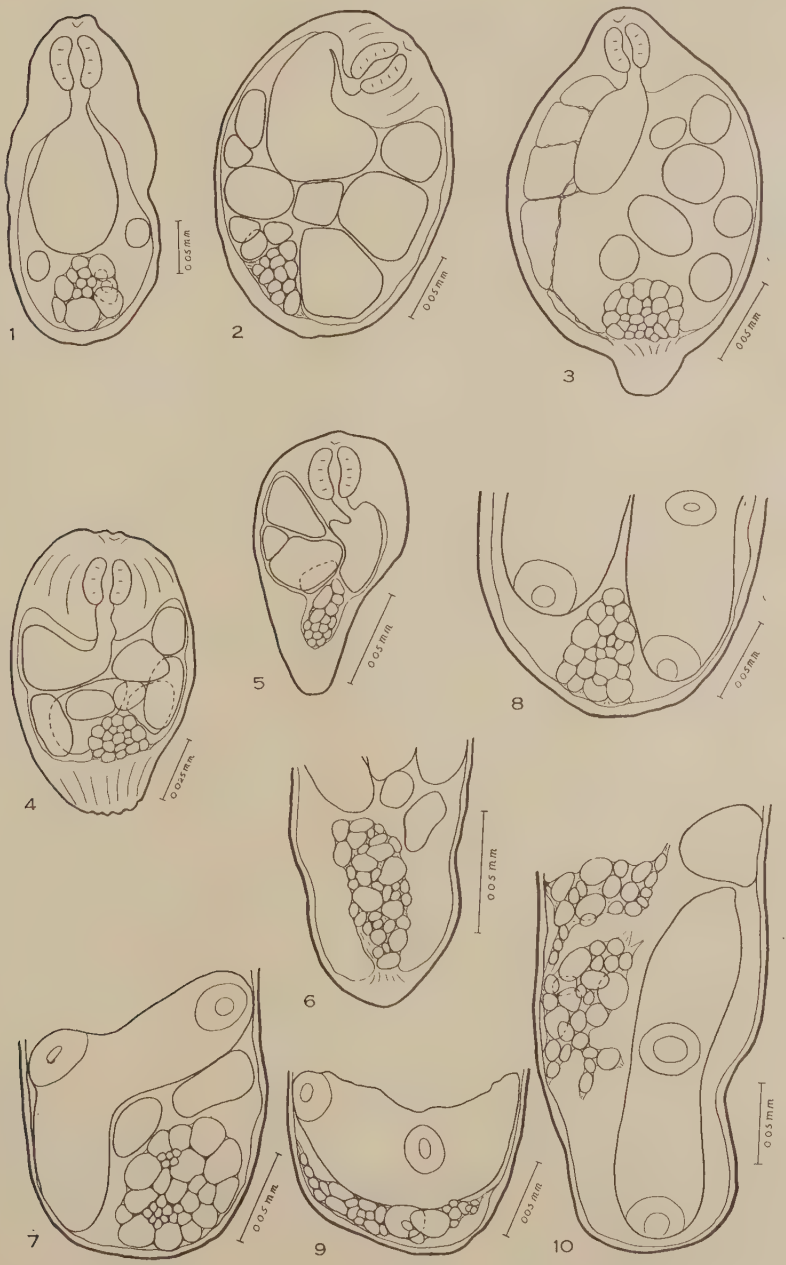
Fig. 7. Posterior end of mature daughter redia from *H. campanulatum smithii.*

Fig. 8. Posterior end of mature daughter redia from *H. campanulatum smithii.*

Fig. 9. Posterior end of mature daughter redia from *H. campanulatum smithii;* germinal mass much flattened.

Fig. 10. Posterior end of old daughter redia, 1.27 mm in length, from *H. antrosum percarinatum;* germinal mass in abnormal position and broken into parts.

PLATE I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TOXICITIES OF SOME ORGANIC CHEMICALS TO *AUSTRALORBIS GLABRATUS*, A SNAIL VECTOR OF *SCHISTOSOMA MANSONI*

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An increased interest in the prevention of schistosomiasis has provided incentive for a renewed search for chemicals to destroy the snail intermediate hosts. Although recent reports have described the molluscacidal properties of dinitro-o-cyclohexyl phenol and its amine salt (Stirewalt and Kuntz, 1947a; McMullen and Graham, 1947) and of benzene hexachloride (Halawani, 1946), very little is known of the toxicity of organic compounds to snails. In order to provide a background for further studies, the molluscacidal activity of a series of 42 organic compounds has been studied. These included alcohols, esters, ethers, aldehydes, ketones, acids, amides, nitriles, amines, and their chlorine substituted products.

MATERIALS AND METHODS

The test snail was *Australorbis glabratus* (Say), intermediate host of *Schistosoma mansoni* in Central America. A laboratory colony of these snails was maintained in 20-gallon aquaria and was fed a constant supply of dried maple leaves and lettuce.

As a routine, 0.01 M solutions of the test compounds were the highest concentrations used in these studies. Materials lethal to the snails at this concentration were retested at greater dilutions until the minimum concentration causing 100 per cent mortality within 24 hours was reached.

Six snails were used for each test at the maximum concentration (0.01 M.) and three for the lower concentrations. A minimum of two additional tests was run to verify the minimum lethal concentration. Snails were immersed for 24 hours in 100 ml. of the solutions in covered finger bowls. Thereafter, they were rinsed and placed in 100 ml. of water from the stock aquaria for an additional 48 hours. The reactions of the snails to the solutions were described only as living or dead. Criteria for death were (a) inability to continue locomotion or adhesion to the glass surfaces, (b) lack of reaction to tactile stimulation, (c) absence of visible heart movement when examined microscopically, and (d) the fading of normal body color to opacity. The effects of the chemicals on the snails were recorded after 24 hours and verified by 72-hour observations. None of the 127 snails exposed to distilled water alone died during the testing.

RESULTS

Twenty (nearly one-half) of the compounds were ineffective as molluscacides at the maximum concentration tested (0.01 M.). These included ethyl alcohol, ethylene glycol diethyl ether, diethylene glycol diethyl ether, paraldehyde, acetone, diacetyl, glycine, acetamide, acetyl glycine, acetyl urea, diethanolamine, triethanol-

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[2] The opinions and statements expressed in this paper are those of the authors and not necessarily those of the Navy Department.

amine, diethyl ethanolamine, ammonium acetate, ammonium oxalate, acetonitrile and ethylene chlorohydrin. Partial effectiveness at this concentration was obtained with acetal which killed 11.5 per cent of the snails and triethylamine which killed 33 per cent.

Unchlorinated acids and their derivatives were more effective (table 1). Oxalic acid was the most toxic compound in this group, killing all of the snails within 24 hours in a 0.004 M. solution. Of the amines tested, ethylenediamine was most toxic with a minimum lethal concentration of 0.005 M. A decrease in toxicity was noted when ethyl groups were substituted for hydrogen atoms on the amino group of ethylamine. Thus, the minimum lethal concentration increased from 0.008 M. for ethylamine to 0.009 M. for diethylamine while triethylamine was ineffective at 0.01 M. Ethyl cyanoacetate, killing in 0.004 M. solution and ethanolamine in 0.006 M. solution were the only other unchlorinated compounds effective at the concentrations tested.

Except for ethylene chlorohydrin which was ineffective at 0.01 M. concentration, all of the chlorinated compounds included in these studies were lethal to snails in solutions ranging from 0.004 M. to 0.0001 M. (table 1). Methyl and ethyl

TABLE 1.—*Compounds which killed all snails in 24 hours in concentrations less than 0.01 M., together with a comparison of the minimum lethal molar concentrations of some of the compounds and their chlorinated derivatives.*

Compound	Minimum lethal molar solution	Index of increase in toxicity by chlorination*
Oxalic acid	0.0040	
Acetic acid	0.0050	
Chloroacetic acid	0.0040	125
Dichloroacetic acid	0.0020	150
Trichloroacetic acid	0.0040	125
Chloroacetyl chloride	0.0030	167
Acetic anhydride	0.0060	
Chloroacetic anhydride	0.0040	150
Ethyl acetate	0.0080	
Ethyl chloroacetate	0.0001	8000
Ethyl acetoacetate	0.0050	
Ethyl cyanoacetate	0.0040	
Methyl chloroacetate	0.0003	
Acetonitrile	**	
Chloroacetonitrile	0.0016	625+
Acetamide	**	
Chloroacetamide	0.0006	1667+
Ethylamine	0.0080	
Diethylamine	0.0090	
Ethylenediamine	0.0050	
Ethanolamine	0.0060	
Chloral	0.0050	

* Based on ratio of minimum lethal molar solutions of $\frac{\text{unchlorinated compound}}{\text{chlorinated compound}} \times 100$

** Ineffective at 0.01 M., maximum concentration tested.

chloroacetates and chloroacetamide were the most toxic compounds studied. Chlorination of acetic acid and acetic anhydride did not appreciably increase the toxicity of the compounds to snails. On the other hand ethyl chloroacetate, chloroacetonitrile and chloroacetamide were markedly more toxic than the unchlorinated compounds.

DISCUSSION

This work was undertaken in an attempt to correlate chemical structure with molluscacidal activity. Nevertheless, the minimum lethal concentration of ethyl chloroacetate (0.0001 M.) was found to approach that of copper sulfate (0.00002 M.) which is commonly used to kill snails (Stirewalt and Kuntz, 1947b). Dinitro-

o-cyclohexylphenol was reported (Stirewalt and Kuntz, 1947a) to kill at a concentration of 2 ppm. (0.000008 M.). Although the compounds described in this report are not practical molluscacides, they do provide data for further investigations.

CONCLUSION

In this series of tests alpha-chloro-esters and amides of aliphatic monobasic acids showed a more marked molluscacidal activity than the unchlorinated derivatives.

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RHIPIDOCOTYLE SEPTPAPILLATA KRULL, 1934 (TREMATODA): THE CERCARIA AND NOTES ON THE LIFE HISTORY*

VERNE B. KNISKERN

Woodhead (1936) reported five species of gasterostome cercariae from the Huron River watershed in the Ann Arbor region of Michigan. Three of these cercariae could be identified with known adult forms; *Cercaria elegans* (= *Bucephalus elegans* Woodhead, 1930), *Cercaria papillosum* [= *Rhipidocotyle papillosum* (Woodhead 1929)], and *Cercaria argi* [= *Bucephalopsis pusilla* (Stafford, 1904)]. Under Prof. Woodhead's direction, the writer began an investigation of the life histories of the remaining two, *Cercaria basi* from *Lampsilis siliquoidea* and *Cercaria scioti* from *Micromia iris*.

Thus far, experimental studies have demonstrated the life history of *Cercaria basi*. Observations on spontaneous emergence and on dissection of mussels revealed that this species infects approximately seventeen per cent of *Lampsilis siliquoidea* collected from the Huron River, especially where it passes through basins of sufficient size to form a marl bottom lake. Encystment of the cercaria was accomplished by exposing small fish such as *Lepomis gibbosus, Semotilus atromaculatus, Micropterus salmoides,* and the common aquarium guppy *Lebistes* sp. The last was admirable for the purpose as its light color and transparency made detection of the cysts in the flesh very easy. Over-exposure to the cercariae resulted in the death of the fish. The metacercariae encyst in great numbers in the caudal peduncle and at the base of the pelvic and pectoral fins. The cysts when viewed *in situ* in the living fish under a dissecting microscope show an opaque, S-shaped excretory bladder through the cyst wall.

A developmental period of twelve to fourteen days in the fish is evidently necessary before the metacercariae are infective to the final host, for attempts at infection with younger metacercariae failed. Metacercariae of at least twelve days of age have been fed to *Micropterus salmoides* and *Lepomis gibbosus*. When *Micropterus salmoides* was used, the infection was successful in but three of eight attempts and in no instance has any eggs been produced although up to twenty-eight days was allowed for development. Metacercariae of the same lots when fed to twelve *Lepomis gibbosus* were recovered from all, at intervals of from twenty-four hours to five weeks. After seven days of development within the pyloric caeca of the definitive host, recovered adult flukes contained eggs which hatched upon contact with water to liberate miracidia.

The adult gasterostomes recovered from experimentally infected fish conform with Krull's (1934) description of *Rhipidocotyle septpapillata,* which he recovered from *Lepomis gibbosus* fed with cysts from the banded killifish, *Fundulus diaphanus* and *Lepomis gibbosus* from the Potomac River near Alexandria, Virginia. Krull's account of the life history is incomplete, lacking information concerning the molluscan host and the cercaria. *Rhipidocotyle septpapillata* is characterized by the possession of a distinct fan-shaped hood bearing seven short, angular papillae. Since the nature of the anterior sucker is the basis for distinguishing the genera and species within the family BUCEPHALIDAE, there is little doubt that the presence

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* Contribution from the Department of Zoology, University of Michigan.

of the seven papillae is a valid criterion for the establishment of the species. This seven-papillated anterior hood is evident in the adult gasterostomes developed from *Cercaria basi,* especially in the older specimens, while alive. The papillae usually contract upon fixation, but are retained by a few and are demonstrable in whole mounts of these individuals.

Krull reported that the flukes matured in from five to seven days, and were eliminated soon thereafter, although he indicated that in one case the infection persisted for eighteen days. The present study has shown that development is seemingly not so rapid and that seven days or more are required for the production of eggs. Even after five weeks the infection was still heavy and many eggs were being produced. It is possible that the metacercariae from natural sources used by Krull were more mature than the experimentally produced metacercariae used in this study.

Nagaty (1937) found a gasterostomatous trematode in a marine fish, *Thynnus thunnia,* from the Red Sea, which measured 3.168 mm in length and 0.363 mm in width, and which possessed the characteristic seven papillated hood on the anterior sucker. He assigned these worms to *Rhipidocotyle septpapillata,* indicating that within the family BUCEPHALIDAE the nature of the head organ is of greater value for species determination than are comparative measurements, since the forms Nagaty found exceeded the measurements given by Krull.

A comparison of the measurements of *Rhipidocotyle septpapillata* Krull and the adult gasterostomes recovered from the feeding of the metacercariae of *Cercaria basi* Woodhead to the same species of fish, *Lepomis gibbosus* follows. Discrepancies between the two series of measurements may be explained by variances in technique.

To summarize, *Cercaria basi* has been shown to be the larval form of *Rhipidocotyle septpapillata* and the life history completed. Cercariae are shed from the fat mucket, *Lampsilis siliquoidea,* encyst in various small fishes, and develop to maturity in the caecal pouches of the pumpkin-seed sunfish, *Lepomis gibbosus.*

COMPARATIVE MEASUREMENTS
in millimeters

	R. septpapillata Krull from *L. gibbosus*	*R. septpapillata** (*C. basi* Woodhead) from *L. gibbosus*		
	Average	max.	ave.	min.
Total length	.938	.697	.515	.340
Width	.195	.272	.221	.153
Length of ant. sucker	.154	.170	.145	.119
Width of ant. sucker	.160	.187	.145	.119
Number of papillae	7	7	7	7
Diameter of pharynx	.052	.049	.045	.041
Length of ant. testis	.135	.131	.086	.061
Width of ant. testis	.124	.086	.069	.053
Length of post testis	.147	.119	.077	.057
Width of post. testis	.143	.091	.061	.041
Length of ovary	.108	.090	.065	.061
Width of ovary	.093	.098	.073	.049

* The above measurements were made on eleven eighteen day old adults fixed in AFA under slight cover glass pressure and stained in Mayer's HCl Carmine.

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GERMINAL MATERIAL IN THE REDIAE OF *CLINOSTOMUM MARGINATUM* (RUDOLPHI)*

W. W. Cort, D. J. Ameel and Anne Van der Woude

INTRODUCTION

Krull (1934) and Hunter and Hunter (1934 and 1935a) determined that the cercaria of *Clinostomum marginatum* is a fork-tailed form that develops in rediae. The cercariae penetrate various species of fish and develop into large metacercariae, popularly known as "yellow grubs," which attain almost adult size. When infected fish are eaten by certain species of water birds, the "yellow" grubs are freed and migrate to the oral cavity of the host where they become sexually mature in a few days. The eggs escape into the water and the miracidia develop and hatch, penetrating into certain species of planorbid snails. Although Hunter and Hunter (1935a) carried through infection experiments, they did not give an accurate account of the structure of the mother sporocyst and the early development of the rediae. However, they (Hunter and Hunter, 1935b) described the miracidium in detail and found that it had four flame cells.

Because of its furcocercous cercaria and the presence of four flame cells in the miracidium, Faust (1939, p. 90) placed *Clinostomum marginatum* in the suborder Strigeata La Rue, 1926. According to Faust's classification, this suborder contains the superfamilies Strigeoidea Railliet, 1919, Clinostomatoidea Dollfus, 1931, and Schistosomatoidea Stiles and Hassall, 1926. Allison (1943) went a step further and considered that *C. marginatum* should be placed in the suborder Clinostomata in the order Strigeatoidea along with the suborders Bucephalata, Strigeata, and Schistosomata. This species differs from all the other members of this order in having rediae as its secondary germinal sacs.

On account of the relationship of *C. marginatum* to the schistosomes and strigeids, it was of special interest to study germinal development in its rediae. We started this study in the summer of 1947 with only a few mature infections. During the first summer we were able to determine only that the daughter rediae of *C. marginatum* did not have germinal masses such as those found in all the other species of rediae that we had previously studied. In the summer of 1948, we were able to study immature as well as mature rediae of this species from both natural and experimental infections and found that the germinal material is organized in small groups of 2 to 5 cells each, which are free in the body cavity.

MATERIAL AND METHODS

Most of the natural infections of *C. marginatum* we had for study were in adults of *Helisoma campanulatum smithii* (Baker) from the shores of Douglas Lake. Mature infections were also found in two large adults of *H. trivolis* (Say). Part

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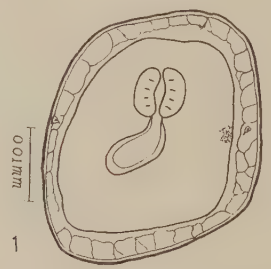
of the mature infections contained a few immature rediae, including some very small ones. We also obtained one immature natural infection in a juvenile of *H. campanulatum smithii* in which there were 18 rediae containing redial embryos. Also, during the summer of 1948 we exposed juveniles of *H. campanulatum smithii* to infection with the miracidia of *C. marginatum*. From these experimental infections we obtained several mother sporocysts each containing one small redia, and free rediae in various stages of development. In one infection rediae were observed which contained both rediae and cercariae. There appear to be three generations of rediae in the life cycle of *C. marginatum*, viz. the single redia produced by the mother sporocyst, the redia-producing rediae that develop in it, and the cercaria-producing rediae found in mature infections.

As in our previous studies on the germinal material in sporocysts and rediae, all our observations were made on living specimens. *Intra vitam* staining with neutral red proved to be particularly helpful in demonstrating the germinal cell groups in rediae of *C. marginatum*.

Germinal Material in the Rediae

In an experimentally infected juvenile of *H. campanulatum smithii* examined 9 days after the last exposure to the miracidia of *C. marginatum* 10 small mother



TEXT FIGURE 1. Mother sporocyst of *C. marginatum* containing a small redial embryo.

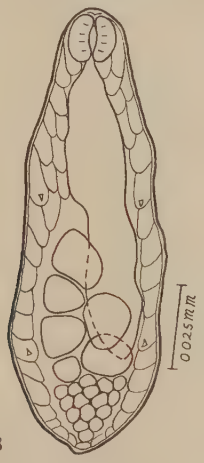
sporocysts were found imbedded in the mantle tissue. Each of them contained a very small redia with the pharynx and intestine already developed (Text fig. 1). A few such rediae were also found outside the mother sporocyst. In some of them,

TEXT FIGURE 2. Very immature first generation redia with germinal cells in its body cavity.

germinal cells could be made out in the primitive body cavity close to the posterior end (Text fig. 2). In another of the experimental snails examined five days later,

an immature first generation redia, 0.138 by 0.046 mm, was found (Text fig. 3). It contained a few free embryos and a number of germinal cells in the posterior end of the body cavity. In snails examined later from the experimental series there were larger rediae in which daughter redial embryos were present. These probably included both first and second generation rediae.

The 18 rediae containing redial embryos which were found in an immature natural infection, mentioned above, were considered to be second generation rediae (Figs. 1 and 2). In them numerous groups of germinal cells could be seen among the developing embryos (Fig. 1). Also, some of the small embryos in these rediae appeared to be attached in groups of two or more as if they had developed directly from the germinal cells of a group (Figs. 1 and 2). Some of the redial embryos were removed from the second generation rediae of this natural infection and carefully studied. In part of them the primitive body cavity in the posterior end con-



TEXT FIGURE 3. Immature first generation rediae, 0.138 by 0.046 mm.

tained only germinal cell groups (Figs. 3 and 4). In others, both groups of germinal cells and a few small embryos were present, and in a few we thought that we saw single free germinal cells (Figs. 5 and 6).

In addition, numbers of immature rediae were studied from some of the mature natural infections. Figures 7, 8, and 9 show some of these rediae which have only germinal cell groups in the body cavity. Others of about the same size, or only slightly larger, contained some free embryos of different sizes in addition to the germinal cell groups (Figs. 10 and 11). Occasionally single free germinal cells were seen (Fig. 11). Also present in certain of the mature infections were a few larger immature rediae, in some of which the germinal cell groups were quite numerous and prominent (Figs. 12, 13, and 14). In a redia represented by figure 14, in which the oldest cercarial embryos were just beginning to be differentiated into body and tail, several groups of germinal cells were seen. Also, in a number of mature rediae that were broken up, search among the freed embryos occasionally revealed germinal cell groups. However, in most of the mature rediae that were examined no germinal material could be found.

It can be seen, therefore, that in the rediae of *C. marginatum* which we examined the germinal cells were almost always found in groups of 2 to 5, although occasionally single cells were seen. The germinal cells in these groups seemed to adhere closely together. We are convinced that the only germinal cells in these rediae besides the very few that appeared singly are those in these groups. We also observed repeatedly groups of two, three or even more small closely adherent embryos, and sometimes germinal cells were attached to such embryos. The germinal cells must be in very rapid division since large numbers of embryos are produced. Our interpretation is that the germinal cell groups are dividing and breaking up into new groups continuously, and that some of the germinal cells are constantly developing into embryos, which either quickly separate from each other or adhere in groups. Some of the germinal cells must retain the ability to divide throughout most of the life of the rediae, because germinal cell groups are still present in some mature rediae which contained large numbers of well developed cercariae and cercarial embryos in all stages of development.

Numbers of rediae and cercariae produced

That the mechanism, described above, in the rediae of *C. marginatum* for the multiplication of germinal cells is very effective is evident from the very large numbers of embryos produced. Rediae were counted in eleven mature infections in adults of *H. campanulatum smithii*, a species which usually has a shell diameter of about one-half an inch. These snails were found to harbor from 1075 to 3131 rediae with an average of 2025. These counts were very difficult to make and are probably less than the actual number. The redial counts for the two infected adults of *H. trivolvis*, which were almost an inch in shell diameter, were 4002 and 4072. We also counted the numbers of cercarial embryos in a series of rediae of different sizes (Table 1). From this table it can be seen that rediae probably begin to give off

TABLE 1.—*Counts of cercariae and embryos in rediae of* C. marginatum.

No.	Length of redia	Cercariae with eyespots	Embryos with divided tails	Smaller embryos	Total
1	0.24 mm	0	7	21	28
2	0.24 "	0	15	29	44
3	0.34 "	3	8	26	37
4	0.48 "	31	16	28	75
5	0.56 "	42	31	51	124
6	0.65 "	55	32	68	155
7	0.66 "	35	43	72	150
8	0.66 "	38	31	58	127
9	0.73 "	43	21	35	99
10	0.97 "	64	44	78	186
11	1.00 "	140	58	111	309
12	1.20 "	160	74	90	324

mature cercariae when they reach a length of about 0.5 mm. The large numbers of mature or almost mature cercariae and developing embryos in the larger rediae indicate that there is a very active multiplication that must continue over a considerable period of time. It is evident that extraordinarily large numbers of cercariae are produced in infections of *C. marginatum*. One infected snail might contain as many as half a million cercarial embryos at one time (2000 rediae each containing 250 cercarial embryos) and during the course of an infection several millions of cercariae would be produced. In fact, the multiplication of individuals in the germinal sacs of *C. marginatum* appears to be greater than for any of the other trematode species we have studied.

DISCUSSION

The mechanism of reproduction in the rediae of *C. marginatum* differs completely from that in all the other rediae we have studied, including representatives of the order FASCIOLATOIDEA and the families HEMIURIDAE, LISSORCHIIDAE, ALLOCREADIIDAE, HETEROPHYIDAE, and TROGLOTREMATIDAE. In all these species germinal masses attached at the posterior end of the body cavity of the rediae serve as centers of multiplication of the germinal cells. *C. marginatum* is related to the schistosomes and the strigeids in which the secondary germinal sacs are daughter sporocysts. The method of multiplication of the germinal cells in its rediae might perhaps be considered as intermediate between that of the schistosomes in which most of the multiplication is by the division and separation of individual germinal cells (Cort and Olivier, 1943; Cort, Ameel, and Olivier, 1944; Olivier and Mao, 1949), and that of the strigeids in which there are numerous floating complex germinal masses which serve as persistent centers for the division of germinal cells (Cort and Olivier, 1941). Perhaps the germinal cell groups in the rediae of *C. marginatum* might be considered as the prototypes of the complex germinal masses in the sporocysts of the strigeids. In fact, they are almost exactly like the early developing stages of these masses as they appear in small daughter sporocyst embryos of the strigeids.

SUMMARY

The germinal cells of the rediae of *Clinostomum marginatum* are in groups of 2 to 5 free in the body cavity. In very small redial embryos only the germinal cell groups may be present. In older rediae they are mixed with the developing embryos. Only occasionally were single free germinal cells seen. It is postulated that these germinal cell groups must be dividing very rapidly to produce the large numbers of embryos that develop in the rediae of this species. It is suggested that they are the prototypes of the complex floating germinal masses of the strigeids.

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Description of Figures in Plate

Rediae of *Clinostomum marginatum* from *H. campanulatum smithii,* showing embryos and groups of germinal cells.

Fig. 1. Anterior end of mother rediae, 0.50 by 0.20 mm; in three or four of the embryos the pharynx and intestine could be made out.

Fig. 2. Mother redia, 0.550 by 0.165 mm, containing several daughter rediae about ready to escape.

Fig. 3. Redial embryo, 0.060 by 0.053 mm.

Fig. 4. Redial embryo, 0.066 by 0.048 mm.

Fig. 5. Redial embryo, 0.075 by 0.042 mm.

Fig. 6. Redial embryo, 0.096 by 0.069 mm.

Fig. 7. Immature daughter redia, 0.063 by 0.038 mm.

Fig. 8. Immature daughter redia, 0.088 by 0.046 mm.

Fig. 9. Immature daughter redia, 0.110 by 0.045 mm.

Fig. 10. Immature daughter redia, 0.070 by 0.045 mm.

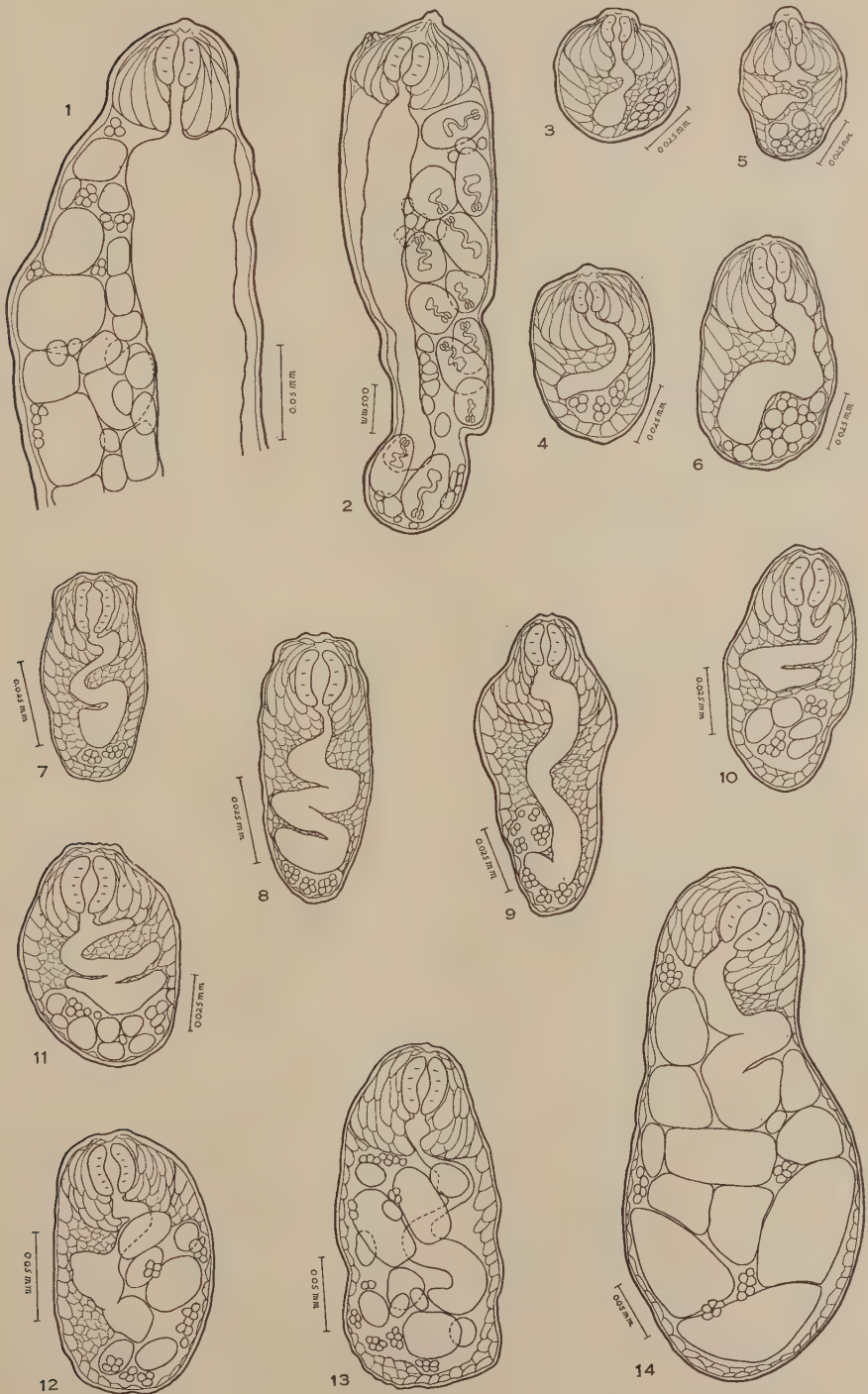
Fig. 11. Immature daughter redia, about 0.075 mm. long.

Fig. 12. Immature daughter redia, 0.150 by 0.080 mm.

Fig. 13. Immature daughter redia, 0.21 by 0.12 mm.

Fig. 14. Immature daughter redia, 0.42 by 0.18 mm, with largest cercarial embryo showing beginning of forked tail.

PLATE I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STUDIES ON FILARIASIS VI. OBSERVATIONS OF THE REVERSAL OF MICROFILARIAL PERIODICITY IN A CASE OF FILARIASIS BANCROFTI[1]

GEORGE W. HUNTER, III[2] AND VIRGINIA G. WARREN[3]

INTRODUCTION

It is well recognized that in autochthonous infections with *W. bancrofti* in Australia, China, India and the Western Hemisphere, microfilariae in the peripheral circulation reach a peak between 10 P.M. and 2 A.M and that few microfilariae occur in the peripheral blood during the daytime.

One of the more obscure aspects of this disease has been the phenomenon of microfilarial periodicity. Observations on diurnal periodicity exhibited in two cases of filariasis bancrofti occurring in American soldiers who contracted the disease in the South Pacific were reported in the fourth paper of this series (Eyles, Hunter and Warren 1947). The present report describes a reversal of periodicity associated with a change in the waking and sleeping habits of an individual who acquired filariasis in British Guiana. This man, whose microfilarial count normally exhibited a nocturnal rise, volunteered to participate in a study which involved determinations of his microfilarial density over a fourteen month period.

CASE HISTORY

A 21 year old negro male was admitted to Walter Reed General Hospital on 9 April 1944 with the diagnosis of filariasis caused by *Wuchereria bancrofti.* He was born in New York City and moved to British Guiana at the age of six years. When he was 16 he noted intermittent swelling and tenderness of the left testicle associated with periods of general malaise and fever. A diagnosis of filariasis was made at this time. The patient also gave a history of having had malaria. At the age of 20 he enlisted in the U. S. Army; he was stationed in Trinidad from March until December 1943. He stated that at this time microfilariae were found in his blood. Upon transfer to Camp Stewart an eosinophilia of 24 per cent was noted. Subsequently he was transferred to Finney General Hospital where a positive intradermal test with *Setaria equina* antigen (1:8000) was obtained; also blood smears were positive for microfilariae and negative for malaria. At Walter Reed General Hospital where the patient was transferred in April 1944 physical examination showed that the right testis, epididymis, cord and vas were normal to palpation. The left testis was enlarged, the epididymis was tender and there was a tenderness on palpation of the vessels but not of the vas deferens, giving the impression of funiculitis, left spermatic cord. Skin tests made shortly after admission with *Setaria equina* antigen were again positive as well as were those with *Dirofilaria immitis* antigen; blood smears, likewise, still contained microfilariae of *W. bancrofti.* Blood counts taken at intervals during the patient's 20 month stay at Walter Reed General Hospital were normal except for an eosinophilia of 24 per cent on two occa-

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TABLE 1.—*Microfilarial counts on patient whose microfilariae normally display nocturnal periodicity*
Counts Represent Number of Microfilariae Per 20 cmm. Whole Blood.[1]

Series number	Date	Slept during	Hour												Mean of series
			6 pm.	8 pm.	10 pm.	12 M.	2 am.	4 am.	6 am.	8 am.	10 am.	12 N.	2 pm.	4 pm.	6 pm.	
I	Aug. 28–29, '44	Day	3	2	1	22	22	105	87	102	93	92	67	10	3	47
II	Sept. 28–29, '44	Day & night	*	8	23	36	80	99	97	72	49	38	6	2	*	39
III	Oct. 26–27, '44	Day	11	4	5	5	5	10	39	48	77	79	75	43	14	32
IV	Dec. 18–19, '44	Ngiht	7	26	45	75	73	46	48	4	2	1	1	*	7	26
V	Feb. 1– 2, '45	Night	6	63	56	85	46	54	36	1	0	0	0	1	5	27
VI	Mar. 13–14, '45	Night	0	43	86	96	47	41	49	1	0	0	0	1	*	28
VII	Apr. 16–17, '45	Night	1	28	53	55	49	43	43	1	*	0	0	*	1	21
VIII	May 9–10, '45	Night	*	29	33	40	48	26	37	4	1	0	0	0	*	17
IX	July 5– 6, '45	Night	6	10	38	50	52	38	31	6	0	0	*	*	*	18
X	Oct. 22–23, '45	Night	2	8	33	37	40	38	30	2	*	0	0	0	0	15
																Mean of all counts
	Hourly mean	Day	7	3	3	13	14	57	63	75	85	85	71	26	9	39
	Hourly mean	Night	3	30	49	63	51	41	39	3	*	*	*	*	2	21

[1] Average counts given as the nearest whole number.
* Less than 1.

sions. During most of the patient's hospitalization at the latter institution he was on "non-strenuous duty," from which he was discharged in December 1945.

MATERIALS AND METHODS

Microfilarial counts were performed every two hours over a twenty-six hour period for a total of ten such series. These covered an interval of fourteen months, during which time the patient either slept during the day and was reasonably active at night (Series I and III); or slept four hours during the day and obtained an additional four hours of sleep during the night (Series II); or slept at night and was active during the day (Series IV through X). Counts were made on 20 cmm. samples of peripheral whole blood prepared as thick films. A Sahli pipette was used and the laked smears were stained with Delafield's hematoxylin. All samples were taken in triplicate and the counts averaged.

RESULTS

The microfilarial counts on this soldier infected with the nocturnally periodic strain of *W. bancrofti,* taken over a period of 14 months, are presented in Table 1. An observation of interest was the finding that although the patient received no therapy throughout his hospitalization the number of circulating parasites showed

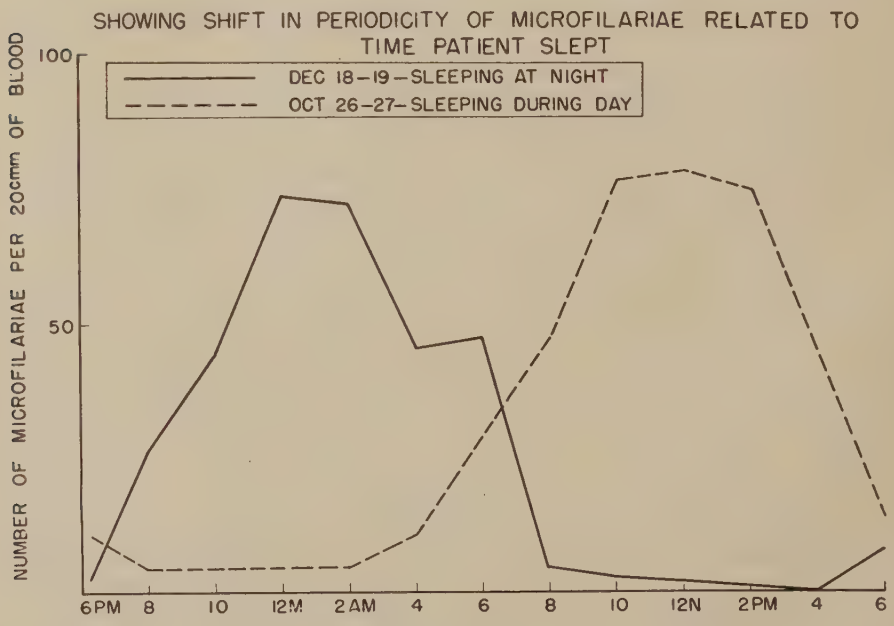

Fig. 1

a gradual decrease from August 1944, when the mean was 46.8 microfilariae for the twenty-six hour cycle to October 1945 when this had dropped to 14.6 microfilariae. The infection at this time was presumed to be over six years old as it was diagnosed in 1939.

Two typical counts are shown in Figure 1. It will be observed that when the patient slept at night and was active during the day (solid line) the microfilarial

count followed the expected curve. It passed its mean about 8 P.M., reached a peak at midnight after which the count decreased, falling below its mean about 7 A.M. until 6 P.M. when there were practically no microfilariae in the peripheral blood.

Upon two different occasions the patient changed his sleeping hours from night to day. Counts were started at 6 P.M., the patient having arisen one hour previously. During the evening and early morning hours the patient engaged in about fifteen minutes of exercise every hour until he retired at 6 A.M. It will be noted that this change in the patient's waking and sleeping intervals caused his microfilarial peak to shift from 12 midnight to 12 noon (see Figure 1). When the patient's sleeping hours were during the day the mean was passed at about 7 A.M., and counts remained above the mean for approximately eleven hours. Around 5 P.M. the circulating microfilariae again decreased to a point below the mean. The patient remained on this schedule for approximately two months during which time on three different occasions additional 20 cmm. samples of blood were taken between 10 A.M. and noon and again between 10 P.M. and midnight. That the reversal in periodicity persisted over this entire period is shown by the contrast between the following day and night counts. In samples taken between 10 A.M. and noon, 75.3, 70.0 and 80.0 microfilariae per 20 cmm. of blood were found, while the evening counts showed only 4.0, 5.0 and 9.0 microfilariae to be present.

Evidence is available that in this particular individual complete reversal of periodicity appeared to conform to the change in sleeping habits. For example, on August 26, 1944, at which time he was sleeping at night, a count of 150 microfilariae per 20 cmm. of blood was obtained at 10 P.M. Beginning August 27 this soldier, by virtue of his duties, was prevented from sleeping at night for several nights. On August 28, 1944 his count at 10 P.M. had decreased to 1.3 microfilariae per 20 cmm. (see Table 1).

One experiment was performed in which the patient was allowed to sleep approximately four hours during the night and a similar amount of time during the day. Microfilarial counts taken during this period showed that this curve fell midway between the other two.

DISCUSSION

Even though the experiments described above are limited to one individual they tend to support the view that periodicity depends upon the hours of activity, rest and sleep; at least in cases dealing with persons infected with *W. bancrofti* showing typical nocturnal periodicity. In contrast, microfilariae found in natives of certain islands of the South and Southwest Pacific are reported (Bahr, 1912) to lack any marked periodicity. An examination of his data suggests that if counts were made at more frequent intervals it is likely that a peak would be reached during the day. Eyles, Hunter and Warren (1947) have suggested that the term "diurnal periodicity" be used to describe this phenomenon which they studied in two patients with this type of periodicity.

Various explanations of microfilarial behavior based on mechanical, chemical and physical phenomena have been advanced. Some investigators (McKenzie 1925) believe that the relaxation of the host's body during sleep may be responsible for the migration of microfilariae to the peripheral blood at night. Such a thesis, however, fails to account for the appearance of microfilariae in the peripheral circulation in individuals infected with a diurnally periodic strain.

More recently it has been suggested that perhaps the microfilariae were attracted by the secretions of the salivary gland of the female mosquito as it fed. Additional indirect evidence for this view is claimed by Highby (1946) who noted that more parasites are recovered from a mosquito's stomach than in a corresponding volume of blood secured by finger puncture. Lane (1929, 1933) has suggested that periodicity is associated with the daily parturition of microfilariae, a theory which seemed to be supported by O'Connor's findings (1931). A possible corollary, that the microfilariae only survive for twenty-four hours, was disproved by both Rao (1933) and Knott (1935), who showed that the microfilariae survived up to two weeks when injected into uninfected volunteers. Furthermore, Knott offered the observation that the nocturnally periodic microfilariae which he used were more sluggish during the day and hence might have been unable to pass the peripheral capillary beds. However, such an explanation would also fail to account for the reversal of periodicity described in this paper.

Our experiments, even though limited and performed on a single individual corroborate the findings of McKenzie that microfilarial periodicity in the nocturnally periodic type is in some way correlated with the period of rest afforded an individual.

SUMMARY

(1) Ten series of microfilarial counts were performed over a period of 14 months on a patient who contracted filariasis bancrofti in British Guiana. Each series represented a twenty-six hour period during which counts were made at two hour intervals.

(2) Nocturnal periodicity was observed when the patient followed normal sleeping and waking habits, i.e., sleeping at night.

(3) Nocturnal periodicity was reversed by altering the sleeping hours.

(4) When the patient slept 4 hours during the day and another 4 hours at night, the microfilarial curve fell between the others.

(5) Although no treatment was administered at any time, the microfilarial counts progressively decreased from a mean of 46.8 per 20 cmm. on August 1944 to 14.6 in October 1945, approximately six years after being diagnosed.

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A NEW SPECIES OF THE ACANTHOCEPHALAN GENUS *OCTOSPINIFER* FROM CALIFORNIA

HARLEY J. VAN CLEAVE[1] AND E. C. HADERLIE[2]

In the course of a survey of parasites of the fishes of Clear Lake, in Lake County, California (Haderlie, manuscript thesis), the Sacramento sucker *Catostomus occidentalis* Ayres, was found to carry a relatively heavy general infection by an acanthocephalan of the genus *Octospinifer*. A preliminary study of stained, permanent mounts revealed that these individuals from the intestine of the Sacramento sucker are in several respects clearly distinct from *Octospinifer macilentus* Van Cleave, 1919, the only previously recognized representative of the genus. Consequently, this material has been made the basis for recognition of a new species which is here described under the name of *Octospinifer torosus*.

Octospinifer was first established as a monotypic genus based upon parasites occurring in the common sucker (*Catostomus commersonnii*) of Douglas Lake, Michigan. In the original description of *O. macilentus*, several distinctive points of anatomical detail were recognized and figured but ready recognition of the genus depends largely upon the number and arrangement of the proboscis hooks. Instead of these being arranged in three transverse series of six hooks each, as diagnostic for the more widely distributed and better understood genus *Neoechinorhynchus*, the proboscis armature in *Octospinifer* consists of three circles of eight hooks each. For most genera of ACANTHOCEPHALA, this difference in number of hooks might fall readily within the established range of individual variability. However, in the family NEOECHINORHYNCHIDAE there is no normal variability in the number and arrangement of the hooks, since these and rudiments of all other structures are laid down with mathematical precision early in ontogeny. Lack of normal variability in these genera is associated with the fixed pattern of the entire developmental process which predetermines in the embryo the number and arrangement of all structures which are to be derived from the cleavage of the fertilized egg. In the very small number of instances where aberrant numbers of hooks have been found in members of this family, the condition is readily recognizable as teratological. Failing to appreciate the stability of conditions in this family, Travassos (1926: 34) regarded *Octospinifer* as a direct synonym of *Neoechinorhynchus*. In his critical monograph of the phylum, Meyer (1932: 170) accepted *Octospinifer* as a distinct genus.

Recently, Van Cleave (1949) reviewed evidences of the differentiation of a distinctive acanthocephalan fauna in the catostomid fishes of North America. The recognition of *Octospinifer torosus* brings to eight the number of species of the family NEOECHINORHYNCHIDAE now described from members of the sucker family on the North American continent. Still further evidence of the differentiation of the NEOECHINORHYNCHIDAE is presented in another paper (Van Cleave and Bangham, 1950), wherein direct emphasis is placed upon the parallel differentiation of acanthocephalan faunas in North American fish hosts.

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In the thirty years since *O. macilentus* was first recognized, there has been no authenticated instance of its occurrence outside a relatively restricted area in the vicinity of the Great Lakes. While some authenticated records fall outside the immediate drainage area of the Great Lakes, in these cases the definitive hosts have been widely distributed species of fishes, often with migratory habits. In a few instances, the incorrectly determined specimens have been reexamined and were found to represent an entirely different species, *Neoechinorhynchus strigosus* Van Cleave, 1949. Introversion or retraction of the proboscis rendered ready identification of these specimens difficult.

In the first extensive survey of ACANTHOCEPHALA of fresh water fishes of our west coast, Lynch (1936) encountered three species of the genus *Neoechinorhynchus* in the western sucker, *Catostomus macrocheilus*, from the state of Washington. Two of these were new species, apparently of restricted geographical distribution, while the third, *N. crassus* Van Cleave, 1919, had previously not been recorded farther west than the states bordering the Great Lakes. More recently, a fourth species (*N. rutili*) has been reported from the west coast (Van Cleave and Lynch, 1949) as an extension of records for the occurrence of this species to include the entire circumpolar area. It is thus apparent that for members of the family NEOECHINORHYNCHIDAE there is no predictable limit to the geographical distribution for the individual species. However, no investigator has previously recorded the finding of any member of the genus *Octospinifer* in fishes of the Pacific coast of America.

Consequently, when specimens of *Octospinifer* were found in suckers of the west coast they aroused more than passing interest. Preliminary surveys have failed to reveal any records of the occurrence of *Octospinifer* in the broad areas intervening between the Great Lakes and the west coast, but sampling has been inadequate over much of this vast territory.

Octospinifer torosus n. sp.
(Figs. 1–5, 7–8.)

Description: With the characters of the genus *Octospinifer* as diagnosed by Van Cleave, 1919; family NEOECHINORHYNCHIDAE, order NEOACANTHOCEPHALA, class EOACANTHOCEPHALA.

Females: 5 to 9.6 mm long, with maximum diameter of 0.5 to 0.89 mm in region of the ventral subcuticular nucleus. Dorsal body wall (Figs. 1–3; 7–8), extremely thickened, often more than 0.2 mm, while ventral wall at the same level is only about 0.035 to 0.07 mm thick. Lacunar vessels conspicuously developed in the dorsal body wall as series of branching tubules (L, Fig. 7). Posterior extremity of females (Figs. 7–8) with a continuation of the greatly thickened dorsal body wall usually forming a bluntly rounded end of the trunk.

Genital pore: (G) subterminal, on ventral surface of the body and usually the thickened dorsal body wall is prolonged ventrally as a small, tail-like projection (Fig. 8). Eggs 0.032 to 0.041 mm long by 0.016 to 0.019 mm; the outer membane frequently incompletely formed.

Males: Usually 3.5 to 6.3 mm in length, with a maximum diameter of 0.5 to 0.8 mm. The dorsal wall of the trunk (Figs. 1, 2) 0.179 to 0.23 mm, thicker than ventral wall (0.03 to 0.045 mm), but frequently with less extensively developed lacunar system than in females. Male genital organs occupy more than the posterior half of the body. The elongately ellipsoidal testes (TA, TP) in fairly broad mutual contact or slightly overlapping. Sperm ducts conspicuous, often with irregular swellings along their course. Syncytial cement gland, containing eight giant nuclei, variable in size, at times as long as the two testes but often much shorter. The cement reservoir, ovoidal with two very conspicuous cement ducts passing posteriorly to the copulatory apparatus. Bursa relatively short when extuded, with comparatively weak development of musculature.

Proboscis: (Figs. 4,5) shortly ovoidal, slightly larger in females (0.120 to 0.158 mm long by 0.158 to 0.198 mm wide) than in males (0.120 to 0.146 mm long by 0.158 to 0.184 mm wide).

Terminal organ, in the axis of the tip of the proboscis, large and inflated, often extending to the level of the posterior circle of hooks. Proboscis armature consisting of three circles of eight relatively delicate hooks each, without conspicuous size differentiation in the three rows; hooks of terminal circle 0.035 to 0.044 mm long; middle circle 0.038 to 0.044 mm; basal circle 0.035 to 0.041 mm. Only the hooks of the terminal circle have a prominently developed, posteriorly directed flat root-process, in some individuals reaching a length of 0.035 mm. Thorns of hooks in terminal and middle circle about 0.008 mm in diameter at the bend near base of thorn; basal hooks more delicate, often only about 0.005 mm in thickness.

Lemnisci in the same individual usually differing greatly in length (Figs. 1–3), in males the longer lemniscus, bearing two giant nuclei, commonly reaches at least to the level of the posterior testis, while the shorter lemniscus, with a single nucleus, is much shorter and of smaller diameter.

Comparisons: Octospinifer torosus differs from *O. macilentus* (Figs. 6, 9), the only other known species of the genus, in having a larger proboscis and somewhat larger proboscis hooks. The dorsal wall of the trunk is extremely thickened in *O. torosus* and the two lemnisci are usually extremely diverse in size while they are but slightly different in size in *O. macilentus* (Fig. 6). The thickened subcuticula of the dorsal trunk wall and the diversity of the lemnisci are distinctly similar to conditions found in *Neoechinorhynchus cristatus* Lynch, a representative of a distinctly different genus within the same family which includes *O. torosus*. The genital region of females of *O. torosus* (Figs. 7, 8) is pronouncedly different from that of *O. macilentus* (Fig. 9).

Abnormalities are fairly frequent in the internal organs of *O. torosus*, involving especially the numbers of subcuticular giant nuclei and the conditions of the male organs. In one male, six dorsal subcuticular nuclei were observed and in another individual there were nine instead of the customary five. Monorchism is not uncommon in this new species and may either involve an apparent fusion of two testes to form a single gonad, larger than a normal testis, or one testis may be wholly wanting. As an intermediate stage, one of the testes may be greatly reduced in size while the other seems to be of normal size.

Type host: Catostomus occidentalis Ayres (Sacramento sucker) of Clear Lake, Lake County, California; limited to about the middle third of the intestine. Incidence of infection very high, from 10 to 20 individuals in each infected host. Only the large suckers, 50 cm in average length, were regularly and heavily infected. Many small individuals of the host species from the habitat where large suckers were heavily infected were entirely free from *Octospinifer* and suckers of intermediate size carried only light infections.

Intermediate hosts and developmental stages wholly unknown.

Holotype male: (VC. accession no. 4272) and paratypes of both sexes deposited in U. S. National Museum. Allotype female and series of paratypes of both sexes in collection of H. J. Van Cleave, Urbana, Illinois; and series of paratypes, both sexes, in collection of E. C. Haderlie, Berkeley, California.

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Explanation of Plate

The morphology of *Octospinifer torosus,* n. sp., compared with *O. macilentus* Van Cleave.

All drawings were made with a camera lucida from permanent mounts stained in borax carmine and mounted in Clarite, Balsam or Piccolite. Katherine Hill Paul, scientific artist in the Department of Zoology, University of Illinois, prepared the drawings and arranged the plate.

Figs. 1, 2, 3 and 6 are of uniform magnification to which the scale between Figs. 2 and 3 applies.

Figs. 4, 5, 7, 8 and 9 are drawn to the same scale, indicated by the line beside Fig. 7.

Symbols

DN1—anteriormost dorsal subcuticular nucleus
E—eggs containing developing embryos
G—genital pore of female
L—lacunar vessel
L1—uninucleate lemniscus
L2—binucleatel emniscus
TA—anterior testis
TP—posterior testis
VN—ventral subcuticular nucleus

Figs. 1–3. Anterior portions of bodies of *O. torosus*, showing relations of proboscis to trunk, diversification of dorsal and ventral walls of trunk and size relations of the lemnisci (L1 and L2), particularly with reference to the location of the testes (TA and TP) and to positions of the ventral giant nucleus of the subcuticula (VN) and the anterior dorsal subcuticular nucleus (DN1).

Figs. 1 and 3. Characteristic paratype male individuals showing the long lemniscus (L2) much larger and longer than the short lemniscus (L1).

Fig. 2. A characteristic mature female paratype showing slightly inflated form of anterior region of trunk. The developing eggs which filled the body cavity were omitted in this drawing.

Figs. 4 and 5. Characteristic proboscides of *O. torosus,* showing arrangement and size of the proboscis hooks and the attachment of the proboscis to the neck and trunk (4, holotype male, 5, paratype male).

Fig. 6.. Anterior end of body of *O. macilentus* male, drawn to same scale as Figs. 1–3, showing contrasts and comparisons with conditions found in *O. torosus*. Observe the smaller proboscis, the shorter lemnisci and the much less conspicuous thickening of the dorsal wall of the trunk. The lemnisci in this species do not extend to the level of the anterior testis.

Figs. 7–8. Posterior extremity of trunk of females of *O. torosus* (7, allotype; 8, paratype), showing ventral location of genital pore (G), excessive thickening of dorsal subcuticular layer of body wall, prominent lacunar vessels (L) and, especially (Fig. 8) the tail-like extension of the body wall ventrally. Eggs (E) in the body cavity, in various stages of development, are scattered among the egg balls.

Fig. 9. Posterior extremity of female of *O. macilentus* showing conspicuous differences between this species and *O. torosus*.

PLATE I